Studies on the Effects of Zinc Ethylenebisdithiocarbamate (Zineb) on Citrus Seedlings Grown in Solution Cultures and Soil and on Its Degradation by Sunlight and Soil Microbial Action

By

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TABLE OF CONTENTS

TABLE OF CONTENTS--Continued

TABLE OF CONTENTS--Continued

TABLE OF CONTENTS--Continued

LIST OF TABLES

LIST OF TABLES--Continued

LIST OF TABLES--Continued

LIST OF TABLES--Continued

Appendix Table Page

LIST OF TABLES--Concluded

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The economical production of agricultural products depends upon the extent to which the producer can control the factors affecting the quantity and quality of his products, and still obtain a profit. Quality control is reached by proper soil management and suitable spray programs to prevent pests or diseases from injurying the crop. Other factors are involved such as choosing varieties that are adapted to the climatic conditions and the complex process of marketing. The effort to achieve such crops often results in a number of problems arising either from insufficient research on the nature of the pesticide being used or the nature of the residues.

In Florida, sulfur sprays, or dusts, became incorporated into the soil and upon oxidation the resulting acidity led to the problem of maintaining soil pH at desirable levels. Another residual soil effect arose from the use of copper sprays or dusts. This resulted in iron chlorosis which was observed on citrus and vegetables growing on soils that had high copper accumulations. Growers assumed previously that the copper leached from these sandy soils. Research became necessary to deal both with the iron chlorosis and the excessive copper availability to the crops. At some expense, use of iron chelates and higher liming programs have now returned such soils to high productivity.

The advent of organic and metal-organic pesticides not only brought about better control of pest and diseases but also more problems were introduced inadvertently concerning the residues from these chemicals. Control of residue levels on produce has received legislative approval. Such residues, however, finally have entered the soil. It may be argued that the soil microbial processes will dispose of the residues by decomposing them to harmless products. Yet, unless these residues in the soil have been tested for their effect on plant physiological processes and on soil fertility, the assumption that they are harmless must only lead to residue problems of the type experienced for sulfur and copper.

The present study was undertaken to evaluate the effect of residues arising from the use of zinc ethylenebisdithiocarbamate, commonly termed zineb. Because widespread use of zineb in citrus groves was introduced about this time, it was decided to use citrus seedlings as the indicator plant in these studies. The objectives were (a) to study the effects of zineb on the growth and chemical composition of citrus seedlings at several copper levels, (b) to determine the effect of zineb exposed to sunlight on the seedling growth and chemical composition, (c) to evaluate the effects of zineb added to soil on the growth and chemical composition of the seedlings, particularly where magnesium and copper levels were varied, and (d) to assess the degree of degradation of the zineb and the dithiocarbamate by solution, soil and microbial studies.

CHAPTER II

REVIEW OF LITERATURE

Zinc Ethylenebisdithiocarbamate

History.--The era of the use of dithiocarbamates as fungicides and disinfectants began with the issuance of a patent to Tisdale and Williams (71) in 1934. This patent contained examples of the derivation of the dithiocarbamates and stated that Cu, Fe, and Mn could be used to form compounds with these derivatives. Nine years later, Hester (33) was granted a patent for fungicidal compounds including the di-Na, Fe, or Cu salts of ethylenebisdithiocarbamic acid, suitable for use on plants or seeds. The di-Na salt was reported by Dimond et al. (16) to show promising results against various diseases of roses, apples, celery and peas. Unfortunately, this salt exhibited lack of stability. Heuberger and Manns (34) increased the stability of this compound by the addition of $ZnSO_4$ and $Ca(OH)_2$. The successful field-testing of this new fungicide by Ruehle (63), in 1945, on late blight of potatoes was the beginning of a research program which yielded voluminous literature over the next fifteen years. In 1950, the American Phytopathological Society adopted (61) the common names of "nabam" and "zineb" for the di-Na and Zn salts of ethylenebisdithiocarbamate, respectively. These common names were accepted by the Committee on Therapeutic Agents and Appliances of the American Veterinary Medical Association, American Medical Association, Association of Economic Poisons Control Officials, American Chemical Society through the Editor of Chemical Abstracts,

and the Insecticide Division of the Production and Marketing and the Bureau of Plant Industry, Soils, and Agricultural Engineering of the Department of Agriculture.

During the last fifteen years zineb has been used in the culture of vegetables, fruit trees, nut trees, grapes, berries, forest tree seedbeds, grain crops, mushrooms, flowers and ornamentals in the United States and Europe. Although it is principally a fungicide, it has been reported as being toxic to the pickleworm (24, 59, 60) and effective against the citrus rust mite (13, 18, 19, 20, 21, 26, 27, 28, 39, 40, 41, 55, 69).

Uses in Florida.--The pioneer work of Ruehle (63) in 1945 was the starting point in many directions for the use of zineb in Florida. During the years following this report, there was extensive work throughout the State evaluating the effectiveness of zineb against certain plant diseases. At the Gulf Coast Experiment Station, Magie was one of the first to report the effectiveness of zineb against diseases of gladioli (48, 49, 50). Large (45) reported excellent results with zineb in controlling pecan scab in experiments at Monticello and Quincy. In experiments conducted in Southeastern Florida, Cox (11) reported that dusts and sprays containing zineb gave good control of the leaf blight diseases of sweet corn caused by *Helminthosporium turcicum* Pass and *H. maydis* Nisikado and Miyake. Cox and Hayslip (12) observed that the increase in *Botrytis cinerea* of tomatoes paralleled the adoption of the dithiocarbamate fungicides in the spray program. At the Subtropical Experiment Station, Conover (10) found that the metal dithiocarbamates were injurious to cantaloupes by causing a premature

leaf drop and reduced the yield of marketable fruit. Of the several materials tested, zineb was less injurious.

A very important discovery was made by Fisher (18, 19) when she was testing zineb against fungus diseases of citrus in 1955. Where zineb was used she observed a sharp decrease in russet, which is caused by the rust mite, Phyllocoptruta oleivora. In 1956, commercial trials were made on Ruby Red, Marsh, and Duncan grapefruit, Hamlin, Pineapple, Valencia and Temple oranges, and tangerines. The zineb was applied at the rate of 1 pound per 100 gallons of water in spray applications between July 2 and August 3. Russet was controlled in every variety tested. In 1957, she reported (20) that russet might be caused by a fungus, Cladosporium brunneo-atrum. After she had sprayed rust mites with a solution containing 0.65 pound of zineb per 100 gallons of water she examined them in the laboratory. It was observed that after the spray had dried the mites began to crawl. Later Johnson et al (41) reported that rust mites were killed on contact by zineb but that sprayed eggs hatched and these young soon died. Other workers (13, 26, 39, 40, 69) confirmed the original recommendation for the use of zineb as a post-bloom and summer miticide for citrus. It was stated by Johnson et al (41) that the half-life of zineb applied alone was only five days compared to zineb with parathion which was 13 days, or zineb and oil emulsion which was 21 days. Their method of analysis was not stated. They also reported in this paper that co-workers at the Citrus Experiment Station had not found a measurable Zn increase in either young or old leaves from the use of zineb sprays.

One of the important changes that resulted from the use of zineb was the indirect benefit from the reduction in the use of sulfur. At the time when zineb came into use, approximately 50,000 tons of sulfur were used annually for the control of rust mite. The acidity produced by each pound of sulfur was stated to require 3 pounds of lime to maintain the soil pH (55).

In the 1960-1961 season, zineb did not prove to be as effective as in previous years. There was some speculation that the rust mite had become resistant to it. This belief did not prevail with all workers; Griffiths (25) attributed the change in zineb affectiveness to mite population differences. Usually, a population peak occurred in July but during that year there was a long period of high population rather than a peak. This rendered zineb less effective since the zineb was usually applied after the peak had been reached.

Decomposition of dithiocarbamates --Ludwig and Thorn (47) reviewed 77 references dealing with the chemistry and mode of action of the dithiocarbamate fungicides. They noted that various products resulting from the decomposition of the dithiocarbamates have been identified. These included ethylenediamine and associated CS_2, ethylenethiourea, ethylenethiuram disulfide, ethylenethiuram monosulfide, and the isothiocyanates. Most of this work was done with nabam but the breakdown process was considered to be similar for other dithiocarbamates. The degradation of zineb in 2.5 N H_2SO_4 was found by Clarke et al (9) to yield ethylenediamine and CS_2. This breakdown has not been studied in soils. However, nabam has been reported (47) to have a half-life of 2, 30 and 96 hours at pH 4, 5, and 6, respectively. They observed that

the decomposition of nabam in the range pH 5 to 6 was found to be oxidative but between pH 4 to 5 became the acid type of decomposition. Rosenthal et al. (62) reported that Zn can be withdrawn from zineb by Cu and the Cu complex formed thereby was highly stable.

Chelation and Structural Properties.--In the reviews by Ludwig and Thorn (47) and Goksøyr (25) it was pointed out that the heavy metal complexes of dithiocarbamates are relatively strong chelate-type of complexes and that this property might apply in very dilute solutions since zineb is rather insoluble. Both the 1, 1 and 1, 2 complexes might be present in zineb as illustrated here.


Zineb


1, 1 Zn Complex


1, 2 Zn Complex

where R designates the remaining part of the molecule.

Other chelates of zinc have been shown to be nutritionally satisfactory sources of zinc for plants in work by Boawn et al. (7), Benson et al. (5), DeKock and Mitchell (15) and Guion and Johns (29). They concluded that not only was Zn uptake affected but that the absorption and translocation of Fe, Cu, Mn as well as Zn was altered. The specificity of the chelate form FeEDTA was reported by Stewart and Leonard (68). The efficiency of metal chelates in plant nutrition was discussed by Martell (52) and their absorption by plant roots by Tiffin et al (70).

Biological Activity of Zineb --To assay fungistatic activity, Manten et al. (51) developed a roll-culture technique utilizing Botrytis cinerea, Penicillium italicum, Aspergillus niger and Rhizopus nigricans. Klopping and Van der Kerk (44), using a similar technique, found that the method of preparation influenced the degree of polymerization of zineb. They noted that when the salt solutions were diluted considerably before mixing that the degree of polymerization was lessened and this resulted in a compound with lower molecular weight. When highly concentrated salt solutions were used a large molecule was produced that was almost inactive as a fungicide. This explained, they said, why mixing of nabam and $ZnSO_4$ in a tank often was noted to be more effective than the manufactured product. Barrat and Horsfall (1) and Barrat et al. (2) showed that the toxicity to fungi could be decreased by lengthening the aliphatic carbon chain or conversion of the secondary amine to a tertiary amine. They also suggested that the toxicity to the fungi by zineb resulted from the precipitation of heavy metals essential to certain enzyme systems of the fungus spore, but considered that the liberation of H_2S in the decomposition of the dithiocarbamate might be the major factor in its toxicity. Klopping and Van der Kerk (44) disagreed with the above suggested mechanisms for the fungicidal activity. They pointed out that the metals associated with the enzyme systems in combination with nabam did possess fungistatic activity and they also concluded that the toxicity of H_2S formed from dithiocarbamate was too low to account for the high fungicidal activity of dithiocarbamates. They suggested that the toxicity was due to the formation in situ of the corresponding diisothiocyanates. They compared the

fungistatic activities of dithiocarbamates and the corresponding diisothiocyanates and took into account the decrease in solubility as the length of the aliphatic carbon chain increased in these evaluations.

Dithiocarbamates and Plant Physiology.--There have been several reports of injurious effects to plants from the use of zineb (10, 73, 74). The injury was usually stunting generally associated with necrotic spots, and was most severe where nabam was used in combination with $ZnSO_4$ and lime.

Other workers have found zineb to give beneficial growth effects. This was attributed to zinc nutrition from zineb (17) with the possibility of nitrogen being utilized as was indicated with ferbam (57).

More recent work by Sato and Yoshizawa (65) using zineb labelled with Zn65 and S35 indicated that either the whole molecule or part of it could be translocated into plants. Distribution of the S35 from zineb applied to the leaves was different however then when it was applied to the roots. Using cucumber roots, they noted absorption of large amounts of S35 where growth was inhibited. They determined radioactive ethylenethiourea with paper chromatography where extraction of the treated leaves was with chloroform.

Earlier, in a greenhouse experiment using a sand-compost mixture for soil, Helleck and Cochrane (30) observed a reduction in the relative numbers of bacteria of the rhizosphere of bean plants after the leaves had been sprayed with zineb. They suggested a translocation of material or breakdown products and loss through the roots to the soil.

Dekhuijzen (14) found, more recently, that when the fungicide, sodium dimethyldithiocarbamate, was taken up by cucumber seedlings that

he could not recover the compound from the plant tissue but he could identify three unknown fungi-toxic compounds which by paper chromatography possessed R_f values of 0.03, 0.27 and 0.58. Kaslander et al. (42) isolated the compound with the R_f value of 0.58 and identified it as the β-glucoside of dimethyldithiocarbamate. The following year, Kaslander et al. (43) isolated the compound with the R_f value of 0.27 and identified it as β-dimethylthiocarbamoylthioalanine. These compounds were likely synthesized within the plant.

Soil studies with zineb.--At the time when zineb was being extensively tested as a fungicide spray for plants, it also was being investigated for its effects on the microbial population of the soil and as a possible agent against soil-born plant diseases. Jacques et al. (38) reported that 2.1 X 10-4 moles of zineb per kg. of soil inhibited nitrification for 12 days and 2.1 X 10-3 moles per kg. of soil caused a lag of 150 days. In these studies, the soil-percolation technique was used with a Fox sandy loam soil. Hildebrand et al. (35) and Moore and Conover (56) found that zineb was not effective against soil-borne diseases of young seedlings. Ranney and Bird (58) showed with cotton seedlings that the degree of effectiveness of the fungicides in the soil varied with temperature. The results of the work of Bird et al. (6) suggested that soil type affected the fungicidal efficiency of zineb in the soil.

Interaction of Magnesium and Zinc

The interaction between Mg and Zn was observed by Merrill et al. (54) while working with tung trees in a Zn-deficient area. When

they added Mg to the soil the Zn content in the leaves increased and when Zn was added to the soil the uptake of Mg was increased. Recent work has been done by Seatz (66) on this interaction. He found that high pH was associated with Zn deficiency when adding lime materials but he also found that the degree of Zn availability could be altered by choosing these materials on their Mg content. He concluded that the similarity in the size of the ionic radii was a factor.

Accordingly he reasoned that Mg^{++} could substitute for Zn^{++} in an insoluble Zn compound thereby releasing Zn^{++} for the plant. He concluded that this interaction might occur within the plant rather than within the soil because essentially the same amount of extractable Zn was obtained either by extracting the soil with 1 N $Mg(OAc)_2$ or 1 N NH_4OAc. Heslop and Robinson (32) reported the ionic radii to be 0.74Å and 0.65Å for Zn^{++} and Mg^{++}, respectively.

Barrows and Gammon (3) reported that the effect of Zn on Mg uptake depended on the relative concentration of these ions supplied to tung trees. They proposed that the Zn has an initial complementary effect on the Mg uptake probably caused by exchange reactions. With increased Zn supply, the Zn competed with Mg on the root surfaces whereby an antagonism between these two ions might occur. A final complementary effect was explained as Mg and Zn substitution in the protein complexes of the root. The Arredondo loamy fine sand used in their experiments was much less subject to the above interaction than was the Lakeland fine sand that they studied.

CHAPTER III

MATERIALS

Soils

Arredondo fine sand.--Soil was selected from a recently limed Hamlin grove in a rolling area about 3 miles southwest of Orange Lake within Marion County. It was a typical Arredondo soil derived from beds of unconsolidated sand with a Red-Yellow Podzolic color profile. The first 8 inches of this profile was a dark brownish grey color with loose single-grained fine sand structure, and was collected for an outdoor lysimeter experiment. It had a pH of 6.95 in water and 6.25 in 1 N KCl with an exchange capacity of 7.1 me. per 100 g. of soil. Fine particles of agricultural limestone could be seen among the sand grains of the collected soil. Mature trees on this soil showed severe symptoms of Zn deficiency, which had been noted since before the addition of lime.

Arredondo fine sand.--A second Arredondo soil was obtained from a cultivated area adjacent to the mature grove described above, which had not been recently limed. This soil had a pH of 6.05 in water and 4.90 1 N KCl and was used also for lysimeter jars in outdoor and greenhouse experiments.

Leon fine sand.--A Leon soil was obtained from a mature Hamlin grove in a level, drained area about 15 miles west of Kissimmee, Florida. Its prominent humus B horizon within 30 inches distinguished it as a Leon which is a Ground-Water Podzol formed from moderately thick beds

of sand. The surface 4 inches of this soil was dark grey in color with a pH of 6.05 in water and 5.55 in 1 N HCl and had an exchange capacity of 4.4 me. per 100 g. The underlying A2 horizon, or leached zone, was much lighter in color with a pH of 5.45 in water and 4.55 in 1 N HCl and had an exchange capacity of 2.5 me. per 100 g. Soil was collected from the surface 4 inches and the first 4 inches of the A2 horizon to reconstruct a profile for a greenhouse experiment. Mature trees on this soil showed prominent leaf symptoms of minor element deficiencies.

Pomello fine sand.--A Pomello soil was obtained from a slight rise within a somewhat poorly drained area about 5 miles west of Gainesville, Florida. This soil was characteristic of this soil type which is derived from thick beds of sand with a shallow A1 horizon of 2 inches and a light grey A2 horizon extending to an organic cemented hardpan at 48 inches. The A2 horizon of this soil was chosen for microbiological studies because it contained only 0.20 per cent organic matter, and was naturally very low in the elements to be determined in the microbiological degradation studies. The pH of this soil was 4.60 in water and 3.75 in 1 N HCl and it had a water-holding capacity of 22.0 g. of water per 100 g. of soil.

Soil Extracts for Inoculations

Soil extracts were made from both the Arredondo fine sand as described in Chapter III and the surface 4 inches of the Leon fine sand as referred to in Chapter XII by shaking 500 g. of each fresh soil in 1,000 ml. of distilled water for 15 minutes, letting stand for

30 minutes, and filtering through cheesecloth. This extract was prepared just before using and was thoroughly shaken just before the withdrawal of each aliquot to reduce differences between aliquots. The percent organic matter in these Arredondo and Leon extracts were 0.0938 and 0.0428 respectively.

Fertilizers

6-6-6 Fertilizer.--A commercial fertilizer derived from ammoniated superphosphate, ammonium nitrate, sulfate of potash-magnesia, Peruvian guano, activated sewage sludge, tobacco stems, tung meal, manganese sulfate and copper sulfate with dolomite as a filler was used in part of the experiments. The complete tag analysis showed 2.0 per cent MgO, 0.25 per cent MnO and 0.20 per cent CuO to be present.

8-2-8 Fertilizer.--A commercial fertilizer of 4-7-5 grade derived from ammoniated superphosphate, ammonium nitrate, nitrate of potash, and manganese sulfate was used with additional nitrogen and potash from ammonium nitrate and potassium sulfate, or sulfate of potash-magnesia, to give 2 fertilizers, each with an 8-2-8 analysis. One contained 6.5 per cent MgO and 0.20 per cent MnO whereas the other did not have MgO present but contained the 0.20 per cent MnO.

Chemicals

General.--All chemicals used in these experiments were reagent grade chemicals unless so stated.

Dithiocarbamates.--Two commercial metal salts of carbamic acid derivatives, zinc ethylenebisdithiocarbamate and di-sodium ethylenebisdithiocarbamate were used. The Zn salt, known as zineb, is insoluble in water and was obtained as a wettable powder with 65 per cent active ingredient and a Zn content of 15.4 per cent. Nabam, the di-Na salt, was obtained in a water solution with 22 per cent active ingredient. The commercial Zn salt as stated above had an exchange capacity of 6.5 me. per 100 g.

Water.--All water used in the analysis, nutrient solutions or in experiments where Zn was to be determined was either deionized or redistilled from glass.

Test Plants

The one-year-old sour orange seedlings (Citrus aurantium) and rough lemon seedlings (Citrus jambhiri) used in these experiments were obtained from commercial nurseries in Polk county, while the sour orange seed were extracted from fruit of trees in Hillsborough County.

Nutrient Solutions

A modification of Hoagland's No. 2 solution (36) was used. It consisted of using some different source materials and deleting the Zn from the minor elements. The components of the solution that were modified are shown in Table 1. All of these solutions and the H_2SO_4 solution were extracted with dithizone in carbon tetrachloride for further purification.

The B, Mn, and Mo solutions were made from H_3BO_3, $MnCl_2 \cdot 4H_2O$ and $H_2MoO_4 \cdot H_2O$ (85% MoO_3) and were used at concentrations of 0.5, 0.5 and 0.05 ppm., respectively, in the nutrient solution.

TABLE 1

Nutrient solution modification

Hoagland's No. 2 Solution		Modification Used	
Source	Molarity of Nutrient Solution	Source	Molarity of Nutrient Solution
$NH_4H_2PO_4$	.0010	$Ca(H_2PO_4)_2 \cdot H_2O$	.0005
KNO_3	.0060	KNO_3	.0060
$Ca(NO_3)_2$	.0040	$Ca(NO_3)_2 \cdot 4H_2O$	.0035
$MgSO_4$	.0020	NH_4NO_3	.0010
		$MgSO_4 \cdot 7H_2O$	.0020

A Cu solution from $CuSO_4 \cdot 5H_2O$ was used to give a Cu concentration of 0.02 ppm. in the nutrient solution except where stated otherwise.

One ml. of a 1.5 per cent solution of $FeSO_4 \cdot 7H_2O$ was added about once a week to each jar as the Fe source.

CHAPTER IV

ANALYTICAL METHODS

Chemical Analysis

Preparation for analysis

Leachates.--The leachate collected from each of the lysimeter jars was filtered through Whatman No. 42 filter paper and a 1,000 ml. aliquot was taken to dryness on a hotplate at a low temperature. The organic matter in these residues was destroyed by treating with 10 ml. of concentrated HNO_3, 5 ml. of 72 per cent $HClO_4$, and taken to dryness at a low temperature. These oxidized residues were taken up twice with 10 ml. of 0.5 N HCl and made to a volume of 100 ml. for analysis.

Leaves.--Leaves were collected from the plants and dried in an oven at 70°C without washing. The entire sample was digested in 100 ml. of concentrated HNO_3, or more if necessary, until a clear light amber color was obtained, then 25 ml. of 72 per cent $HClO_4$ was added and the samples were taken to dryness at a low temperature. The residue was taken up twice with 10 ml. of 0.5 N HCl and made to a volume of 100 ml. for analysis.

Roots.--The roots of the solution culture experiments were washed with a strong stream of tap water followed by a rinsing with deionized water. They were dried at 70°C, and the entire root system was digested and made to volume as described for leaves above.

Roots of the seedlings growing in soil were placed on a plastic

screen to catch any roots that might become detached subsequently in the washing process. After the soil was washed free by a strong spray of tap water, the roots were rinsed with deionized water. These roots were also dried at 70°C., digested, and made to volume as described above.

Soils.--Soils used in the microbiological experiments were extracted with a 10 per cent NaCl solution adjusted to either pH 2.5 or 4.0 for Zn analysis. A 50-ml. portion of this extracting solution was added to 10 g. of fresh soil, which was calculated on the dry weight basis, in a 100-ml. polyethylene centrifuge tube, stoppered, and vigorously shaken for 15 minutes. After centrifuging, the supernatant was decanted and used for subsequent analysis.

Soil samples from the lysimeter experiment were extracted with 50 ml. of 0.1 N HCl for Zn analysis by shaking 10 g. of dry soil for 30 minutes and filtering through a Whatman No. 42 filter paper.

Extracts for NH_4-N and SO_4-S were obtained by shaking 25 g. of fresh soil, calculated on a dry weight basis, with 50 ml. of a 10 per cent NaCl solution at pH 2.5 in a 100-ml. polyethylene test tube for 30 minutes. Aliquots were taken for NH_4-N and SO_4-S from the supernatant after centrifuging.

The NO_3-N was extracted by using a modification of the procedure described by Jackson (37). Ten g. of soil were shaken for 10 minutes with 50 ml. of 0.02 N $CuSO_4$ and 0.004 N Ag_2SO_4 in a 100 ml. polyethylene test tube. Then 0.1 g. of $Ca(OH)_2$ was added and the samples were shaken for 5 more minutes. This was followed by the addition of 0.2 g of $MgCO_3$ and shaken for 5 minutes again. After centrifuging, the clear solution was used for the determination of NO_3-N as described below.

Analytical procedures

Calcium --Calcium was determined on a Beckman DU flame photometer at a wavelength of 423mμ and a slit setting of 0.06 using a H_2-O_2 fuel system. The compensating solution was made by adding 32.8 g. of ethylenediamine tetraacetic acid (EDTA), 3.64 g. of $NH_4H_2PO_4$, 4.12 g. of $(NH_4)_2SO_4$, and 6.2 g. of H_3BO_3 to 900 ml. of distilled water, adjusting the pH to 8.6 with NH_4OH, and diluting to 1,000 ml. Two ml. of this compensating solution were added to a flame beaker containing 2 ml. of the sample. Calcium emission was compared to standards prepared in like fashion with 0, 50, 100, 200, 250 ppm. Ca present.

As indicated in appendix table 1, this method gives slightly lower results than the Versene titration method (37) but it has the advantage of being relatively fast.

Carbon dioxide --The absorbed carbon dioxide from the microbiological degradation studies was precipitated as carbonate by the addition of 10 ml. of a 20 per cent $BaCl_2$ solution to the 0.5 N NaOH absorbing solution. The excess NaOH was titrated with 0.1 N HCl using phenolphthalein as the indicator. From the difference between this titration and the original NaOH present the mg. of C in the CO_2 were calculated:

$$\text{mg. of C} = 6 \times \left[(\text{ml.} \times \text{N of NaOH}) - (\text{ml.} \times \text{N of HCl})\right]$$

Copper --Copper was determined by the biquinoline method (8). An aliquot of the sample was placed in a 125-ml. separatory funnel and 15 ml. of an NH_4OAc buffer, at pH 4.8, and 1 ml. of a 10 per cent $NH_2OH \cdot HCl$ solution were added and mixed well. The pH was adjusted to the range of 4.0 to 4.5, using a 50 per cent NH_4OH solution. Ten ml. of a

0.02 per cent 2, 2' biquinoline solution in iso-amyl alcohol were added to the separatory funnel and shaken vigorously for 1 minute. After the layers had separated, the organic phase was transferred to a 15-ml. centrifuge tube and placed in the refrigerator for 15 minutes before centrifuging. A standard curve containing 0, 5, 10, 20, and 30μg of Cu was obtained in the same manner as the samples. The samples were read at a wavelength of 540mμ on a Beckman Model B spectrophotometer.

Iron.--Iron was determined by the 1, 10-phenanthroline method (64). An aliquot was transferred to a 50-ml. Pyrex beaker, and 1 ml. of 1 per cent hydroquinone solution and 1 ml. of an aqueous saturated solution of 1, 10 phenanthroline were added. Twenty ml. of a 25 per cent solution of sodium citrate, containing 50 ml. of concentrated HCl per 1,000 ml. were added which resulted in a pH of 3.5 to 4.0. A standard curve was prepared from 0, 10, 20, 40, and 60μg of Fe in like fashion. The samples were mixed and allowed to stand for 30 minutes before reading at 510mμ on a Beckman Model B spectrophotometer.

Magnesium.--Magnesium was determined on the same aliquot used in the Ca determination at a wavelength of 371mμ and a slit setting of 0.15 with the same fuel system. A standard curve of 0, 15, 40, 85, and 150 ppm. Mg was prepared and analyzed in the same manner as the samples. It can be seen in appendix table 2 that this method agrees very well with Mehlich's colorimetric method (53).

Manganese.--Manganese was determined by the periodate method (64). An aliquot was transferred to a 100-ml. beaker containing 1 ml. of concentrated H_2SO_4 and taken down to SO_3 fumes. Seventy ml. of a solution containing 5 per cent HNO_3, 3 per cent H_3PO_4, and 0.1 per cent

$AgNO_3$ were added. Approximately 0.3 gram of potassium meta-periodate was added to the mixture after it had cooled slightly from being heated to 70°C. for 30 minutes. The beaker was covered with a watch glass and heated at 80°C for 1 hour, cooled, and made to a volume of 100 ml. The samples were read at 525mμ using a Beckman Model 9 spectrophotometer. A standard curve was prepared from 0, 50, 100 and 150μg. of Mn and treated in the same fashion as the samples.

At times this method was modified to allow for final volumes of 10, 25, and 50 ml. for the determination of smaller quantities.

Nitrogen.--For NH_4-N, the Nessler Method was used with the reagents prepared as described by Jackson (37). An aliquot was transferred to a 25-ml. volumetric flask and 2 ml. of a 10 per cent sodium tartrate solution were added. The volume was made up to about 20 ml. and, while shaking, 1.25 ml. of Nessler's Reagent was added rapidly. The samples were made to a 25 ml. volume, mixed, and after standing for 25 minutes, were read at a wavelength of 410mμ. using the Beckman Model 9 spectrophotometer. A standard curve using 0 to 50μg. of NH_4-N was used in the calculation of the amount present in the sample.

To overcome the interferences from the products derived from the addition of high concentrations of sucrose to some soil samples, a modification with permutit (23) was used. Approximately 0.5 g. of permutit was measured into a 25-ml. volumetric flask and washed with about 10 ml. of distilled water to eliminate the fine particles. After the fine particles were poured off, an aliquot of the sample was added and the mixture was shaken for 5 minutes. The permutit was washed to the bottom

with distilled water and allowed to settle, then the liquid was decanted from it. It was washed twice more with 10 to 15 ml. of distilled water, being careful that none of the permutit was lost. To the washed permutit, 2 ml. of distilled water and 1 ml. of 10 per cent NaOH were added, shaken well, and let stand for 5 minutes. The samples were diluted to about 20 ml. and the Nessler method was continued as described in the previous paragraph.

The NO_3-N was determined by the nitrophenoldisulfonic-yellow color method (37). An aliquot of the clear soil extract obtained as described in Chapter IV was taken to dryness in an 8-cm. evaporating dish and cooled. Three ml. of phenoldisulfonic acid were added rapidly to the residue in the evaporating dish and allowed to react for 10 minutes. The resulting mixture was diluted with cold water and neutralized with 5 N NH_4OH, as indicated by the appearance of a bright yellow color, then a few drops in excess were added. The sample was diluted to a 50-ml. volume, mixed, and read at 420 mμ with a Beckman Model B spectrophotometer.

In samples where high concentrations of sucrose had been added, it was necessary to use activated charcoal to remove the charred organic matter after the samples were made to a 50-ml. volume.

Phosphorus --A spectrophotometric method using the 1-amino-2-naphthol-4-sulfonic acid reagent (37) was used for the determination of P. An aliquot of the sample solution was transferred to a 50-ml. volumetric flask and 20 ml. of distilled water were added. This was followed by 5 ml. of a 2.5 per cent solution of ammonium molybdate in 5 N H_2SO_4 and 1.5 ml. of 1-amino-2-naphthol-4-sulfonic acid reagent

This was prepared by adding 7.5 grams of the reagent mixture dissolved in 47 ml. of distilled water. The sample was made to volume and mixed. Transmittance was compared to standards prepared in the same manner containing 0, 20, 40, 60, 80, and 100 ug. of P at 660 mu using the Beckman Model B spectrophotometer in the interval of 5 to 15 minutes after preparation.

Potassium.--Potassium was determined on the same aliquot used for Ca with the flame photometer at a wavelength of 768 mu and a slit setting of 0.06 with the same fuel system. A standard curve was prepared in the same manner with 0, 50, 100, 200, and 300 ppm. of K.

Sulfates.--A turbidimetric method (4) utilizing an excess of sized $BaCl_2$ crystals was used to determine SO_4-S in the soil samples. A 50-ml. aliquot was concentrated down to 20 ml., 10 ml. of which was used for the sulfate determination while the other portion was used as a blank. To the portion that was used for the sulfate determination, 1 ml. of 6 N per cent HCl solution and 1 g. of 30-to 60-mesh $BaCl_2$ were added. The samples were shaken for 5 minutes and read within the next 25 minutes at a wavelength of 465 mu on a Beckman Model B spectrophotometer. A standard curve prepared in the same fashion with 0, 25, 50, 75 and 100 ug of S was used.

Zinc.--The dithizone method (64), using sodium thiosulfate as a complexing agent, was used to determine Zn. A 5-ml. aliquot, or less, was transferred to a 20-ml. vial with a polyethylene top. One drop of a 0.001 per cent solution of chlorophenol red indicator was added and the acidity was adjusted with 3 N NH_4OH to an approximate pH of 5.0. Two ml. of a 1 N NaOAc solution buffered at pH 4.8 and 2 ml. of a 25 per cent

$Na_2S_2O_3 \cdot 5H_2O$ solution were added and mixed. Five ml. of a 0.002 per cent dithizone solution in CCl_4 were added and the sample was shaken vigorously for 3 minutes. The aqueous layer was removed and the organic layer was read at 535mu or 620mu with a Beckman Model B spectrophotometer. Precautions were taken to perform this analysis in a darkened room. A standard curve was prepared similarly with 0, 1, 2, 3, 4, and 5ug of Zn.

Zinc ethylene bisdithiocarbamate.--The method of Clarke and coworkers (5), with the addition of disodium EDTA as used by Levitsky and Lowen (46), was used for the determination of dithiocarbamate. The sample of zineb was placed in a distilling flask with 200 ml. of 1 N EDTA and connected in series to 2 scrubbers containing 30 per cent $Pb(OAc)_2$ and a scrubber of the absorbent for CS_2 as described by Viles (72). The solution was heated just to boiling, then a gradual suction was applied as 60 ml of hot 10 N H_2SO_4 was slowly added to the mixture. The evolved CS_2 in the absorbent gave a yellow coloration corresponding to the quantity of CS_2 released. The samples were compared to a standard containing 0, 30, 100, 150, and 200ug of zineb analyzed in similar fashion. The samples were read at 380mu using a Beckman Model B spectrophotometer.

Organic Matter.--Organic matter, designated as O. M., was determined by the chromic acid oxidation method (37). Two g. of soil were weighed into a 500-ml. Erlenmeyer flask and 10 ml. of 1 N $K_2Cr_2O_7$ were added. Twenty ml. of concentrated H_2SO_4 were added, mixed thoroughly, and the samples were allowed to stand for 30 minutes. The samples were diluted with 150 ml. of distilled water and 10 ml. of H_3PO_4

were added. Six drops of a 0.025 M o-phenanthroline-ferrous complex as indicator were added and the samples were titrated to a red end-point with a 1 N solution of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$. The per cent organic matter was calculated as follows:

per cent O.M. in soil = ml. of $K_2Cr_2O_7$ x 0.345.

Exchange capacity.--The exchange capacity was determined using the method of Jackson (37). Ten g. of soil were saturated with NH_4-ions using neutral 1 N NH_4OAc, then, the NH_4-ions were removed with a 10 per cent solution of KCl adjusted to pH 2.5 with HCl. This solution was made alkaline and the NH_3 was distilled into a 4 per cent H_3BO_3 solution and titrated back to its original pH with 0.1000 N HCl, using a mixed indicator of 124 mg. of methyl red and 82 mg. of methylene blue in 100 ml. of ethanol. The exchange capacity was calculated as:

me. per 100 g. of soil = ml. of 0.1000 N HCl titration x $\frac{100}{10}$

pH.--The pH in water was determined by weighing 50 g. of air-dry soil, calculated in the case of fresh samples to a dry basis, and adding 100 ml. of distilled water. The samples were stirred intermittently and read at the end of an hour on a Beckman Zeromatic pH meter.

The pH determination in a 1 N KCl solution was treated as above with 1 N KCl being used in place of the distilled water.

Infrared Analysis

Three 30 ml. aliquots of leachate were placed in a weighing bottle and taken to dryness at 107°C. After drying, the weighing bottle was placed in a desiccator until the infrared analysis was made.

The analysis was done on a Perkin-Elmer Model 21 infrared spectrophotometer using the KBr mull technique.

X-ray Diffraction Analysis

A 50-mg sample of zineb powder at different exposures was mixed with 2 ml of a 10 per cent solution of glycerol, agitated thoroughly and spread over a petrographic slide. This was allowed to dry for several days and taken for X-ray diffraction analysis to the Chemistry Department. The analysis was made on a Norelco Geiger-counter X-ray spectrometer according to the method described by Fiskell et al (22).

Physical Measurements

Dry weights --After the material had been dried to a constant weight at 70^{o}C , the dry weights of large samples were determined using a torsion balance, but, for small samples an analytical balance was used. The length of time of drying varied with the material.

Root volume --Root volume was determined by the displacement of water by the root system For small root systems a 100-ml. buret was used, and a larger buret was used when necessary.

Soil moisture --Soil moisture was determined by weighing approximately 50 g. of moist soil before and after drying at 110^{o}C. The moisture was expressed in percent and calculated on the basis of the dry soil.

Water holding capacity --The water holding capacity was determined by placing approximately 30 g. of soil into a porcelain Gooch crucible and adding water until thoroughly wet. The air bubbles were removed by stopping the water drainage and tapping the side while water remained

above the surface of the soil. Fifteen minutes after water had ceased to drain from the crucible, it was blotted with a filter paper and the moist soil was transferred to another crucible to determine the water content by difference in weight before and after drying at 100°C.

Tissue Sections[1]

Fixing.--Root tissue was fixed by placing it in a 20 ml.-vial and adding enough of Craf modified fixative to keep it covered. The fixative solution was prepared by combining equal parts of two components, A and B, just before using. Component A was made from 1 g. of CrO_3 crystals, 7 ml. of HOAc and 92 ml. of distilled water. The B component was prepared from 30 ml. of formalin and 70 ml. of distilled water. The root tissue remained in this fixative solution for 24 hours.

Dehydration.--The root tissue was removed from the fixative solution and washed well with distilled water. Dehydration was obtained by placing the tissue for an hour in each of the solutions listed in appendix table 3. The sequence was the same as in shown in the table.

Imbedding in paraffin.--After removal from the pure tertiary butyl alcohol, the tissue was placed in a 1:1 mixture of tertiary butyl alcohol and melted paraffin and heated in an oven at 60°C, for about 48 hours. It was removed from this mixture and orientated in a 1-inch cubical paper container that had been filled with melted paraffin which had started to solidify on the bottom from being placed on a cold surface.

[1]This procedure was outlined and supervised by Mr. Marvin Alvarez, a graduate student in the Department of Botany at the University of Florida.

This container was floated in ice water until the surface of the paraffin hardened, then it was placed under the ice water and held there for about 30 minutes.

Sectioning.--The container was removed and the paraffin block was trimmed to within 3 mm. of the tissue. This trimmed paraffin block was attached to a block of wood with melted paraffin and mounted in a microtome for alignment. The tissue was sectioned at 10 to 15μ in thickness and fitted on slides that had been treated with a film of Haupt's adhesive (31) and wet with 3 per cent formalin. These slides were then placed on a warming plate at 40°C. for an hour and allowed to dry in a cool, dry, dust-free place for 24 hours.

Staining.--For this tissue, a staining series containing fast green, safranin, tannic acid, and ferric chloride was used. The solutions were placed in Coplin jars and staining was done in series according to the steps outlined in appendix table 3.

Mounting.--While the slide was still wet with xylene, one drop of Permount was placed on the slide and the cover slip was applied, being careful not to trap air underneath. A weight was placed on the covered slide and left for 2 to 3 hours. The final step was the examination of the slides to see if they were acceptable or not

CHAPTER V

EXPERIMENTAL PROCEDURE

Effect of Zinc on Rough Lemon Seedlings Grown in the Presence or Absence of a Toxic Concentration of Copper in a Solution Culture

An experiment with rough lemon seedlings was conducted to determine what effects zinc would have with or without a concentration of copper in the solution culture that was likely to be toxic to the roots. This experiment was conducted in the greenhouse with 30 wide-mouth brown glass jars containing 2,000 ml. of the nutrient solution described in Chapter III, and the rough lemon seedlings as discussed in Chapter III. Previously these jars were washed with a detergent, rinsed with tap water, rinsed with concentrated H_2SO_4 soaked 24 hours with 10 per cent H_2SO_4, rinsed with tap water, and given a final thorough rinsing with distilled water. Air was supplied to these jars from a compressor and an air tank where the air pressure was maintained between 40 and 65 pounds per square inch at all times. A regulating valve was located at the lead-off from the tank for adjustment of the air flowing from the tank through a manifold to each jar. At each jar a screw clamp was used to adjust the continuous air flow leading from a rubber tube to a glass tube extending to the bottom of the jar. The air was dispersed in fine bubbles as it passed out four pin holes in a polyethylene cap at the end of the glass tube. These glass tubes were held in place by a hole in a piece of wax-covered

cardboard that also held the test plant which was in turn supported by a piece of polystyrene foam.

The experimental design was a completely randomized 2 x 5 factorial with two levels 0 and 0.5 ppm. of Cu applied and five levels of zineb containing 0, 1, 7, 13, and 19 ppm., respectively, of Zn. Each treatment was replicated three times. Copper sulfate ($CuSO_4 \cdot 5H_2O$) was used as the source material for the copper treatments and the commercial product described in Chapter III was used as the source material for zineb.

The fresh weights and root volumes of the 1-year-old rough lemon seedlings were recorded before they were transferred to the jars on February 26, 1962, and the treatments were added to the nutrient solutions immediately thereafter. One-half of the nutrient solution was applied at the beginning of the experiment with the other half being applied 3 weeks later. This nutrient solution was not changed during the entire experiment, but the pH of the solutions was checked with an indicator paper and adjusted to pH 5.6 with a saturated solution of $Ca(OH)_2$ when necessary. The jars were made to volume with distilled water once weekly at the beginning of the experiment, but this was increased to every 2 days as the seedlings grew.

After 17 weeks the seedlings were removed and arranged according to the treatments and notes were taken on the visible differences among the tops and roots. The fresh weight of the entire plant, fresh weight of new growth, fresh weight of new leaves, and root volume were recorded for each seedling. The new leaves and roots were prepared and analyzed as stated previously in Chapter IV.

Effect of Zineb, Exposed Zineb and Zinc Sulfate
on Sour Orange Seedlings in a Solution Culture

Zineb, exposed zineb, and zinc sulfate were used in an experiment to compare the effects of these materials at different rates on sour orange seedlings grown in similar solution culture. In this experiment, the equipment and apparatus were the same as described in the previous Chapter, with the exception that 15 more jars were added to accommodate the additional treatments and replications. Also the length of aeration was reduced from a continuous system to the use of aeration for only 4 hours each morning.

Sour orange seedlings described in Chapter III were used as the test plants and were selected according to their visual uniformity of size and stem diameters for each replication. These seedlings were grown in the nutrient solution as given in Chapter III for two weeks before the treatments were applied. This nutrient solution was changed and the treatments described below were added to the new solution on November 15, 1961.

The experimental design was a randomized block with the 3 source materials above applied at rates of 0, 1, 7, 13, and 19 ppm. Zn. Each treatment was replicated 3 times with the commercial product described in Chapter III, being used as a zineb source for both the zineb and exposed zineb treatments and $ZnSO_4 \cdot 7H_2O$ being used as the other source. The exposed zineb used was prepared previously by making a paste of 2.5 g. of the commercial product and spreading it out into a thin layer over the surface of a watchglass having a diameter of 185 cm. This thin layer was allowed to dry and then it was exposed to sunlight for 112 hours.

The seedlings were removed from the solutions on February 18, 1962 and the visible differences in growth were noted. The weights of the new growth and roots were recorded and the leaves and roots were prepared and analyzed as stated earlier in Chapter IV.

Effect of Exposure to Sunlight on Zineb

The effect of sunlight on the stability of zineb was studied by exposing zineb to the sunlight for different periods of time and analyzing the exposed material by chemical, x-ray diffraction and biological analyses.

The exposed material was prepared from the same commercial product and in the same manner as described previously in Chapter V with the exception that the exposures were 8, 32, 56, or 112 hours of sunlight.

Chemical analysis for stability.--The exposed and non-exposed zineb materials were chemically analyzed by the method given in Chapter IV.

X-ray-diffraction analysis for stability.--Samples of the exposed zineb materials were analyzed by x-ray diffraction as described in Chapter IV. Another sample of zineb was washed 10 times with chloroform to determine if the diffraction pattern of the inert carrier could also be ascertained. A known weight of this sample was ashed at 400°C for 5 hours. The weight of the ash was found. A portion was taken for x-ray analysis as before. An exact weight of the ash was dissolved in 0.1 N HCl and analyzed for Zn by the previously described method. Differences in the x-ray patterns before and after ashing were used

to determine the diffraction spacings of the zineb and the carrier. The various Zn compounds in the ash of the carrier gave spacings not observed previously in the zineb patterns.

Biological analysis for stability.--The purpose of this experiment was to test for differences between the effects on rough lemon seedlings from zineb exposed to sunlight for different periods of time. The equipment and apparatus used for this experiment was the same as that described previously in Chapter V. However, the air manifold was redesigned to supply two groups of 24 jars each. Each group of jars was used for a separate experiment which differed only by the level of zineb used.

A completely randomized experimental design was used for each experiment conducted, one being at levels of 7 ppm. Zn from zineb and the other with 13 ppm Zn as zineb. The treatments were zineb exposed 8, 32, 56, and 112 hours, Zn supplied as the sulfate source and a check without Zn. There were 4 replications of each treatment.

Rough lemon seedlings described in Chapter III were selected for each replication according to their stem diameters, and each seedling was weighed and the root volume measured before placing in the jar with the treatment. On February 26, 1962, these seedlings were placed in the jars containing the treatment in 2,000 ml. of the nutrient solution. This solution was not changed during the entire experiment. The pH was checked with indicator paper and corrected by the addition of saturated solution of $Ca(OH)_2$ when needed. Solution levels were adjusted to the original volumes of the experiment and it became necessary to do this more often as the plants grew.

On June 25, 1962, the seedlings were removed from the treatment jars and grouped according to the treatments for observation of the new growth and root systems. After this, the entire plant, fresh weight of new growth, fresh weight of new leaves, number of new leaves, and root volume were recorded for each seedling. The leaves and roots were prepared and analyzed as described in Chapter IV.

Zineb in the Soil

Microbiological experiment 1.--This experiment was designed to evaluate the degradation of zineb in the soil by determining the increased concentrations of available Zn, NH_4-N, NO_3-N and SO_4-S after incubation of zineb with the soil. The apparatus for this experiment consisted of a series of 36-reaction units utilizing a closed system for absorbing the NH_3 or CO_2 evolved. Air from a compressor was supplied to these units after it had been passed through 2 scrubbers containing 4 per cent H_3BO_3, 3 scrubbers containing 40 per cent NaOH and a final scrubber with 4 per cent $Ba(OH)_2$. Each reaction unit consisted of a 500-ml. flask connected by rubber tubing to a glass tube extending to the bottom of a 50-ml. test tube containing the absorbant. A 50-ml. test tube was inserted in the line between absorbant series to prevent the next solution from being drawn back into the reaction flask if the air pressure decreased. The air flow of this system was adjusted by a screw clamp on the open end of a Y-connection inserted in the line in front of the scrubbers. This series of reaction flasks was located in a room where the temperature was 70 to 75°C. at all times. The absorption trains were mounted between shelves holding the treatment flasks.

A completely randomized experimental design consisting of 3 soil variations and 3 materials with 4 replications was used. The soil variations consisted of the A2 horizon of the Pomello soil, Chapter III, and the A2 horizon of the Pomello inoculated with Arredondo or Izon soil extracts, Chapter III. The 3 materials were a medium without added Zn, N, or S, zineb, and a medium containing Zn, N, and S equivalent to that in zineb.

The Pomello soil was screened through a 16-mesh plastic screen, mixed and placed in a plastic container and tightly covered with wax paper. A moisture determination was made on the soil. Soil equivalent to 300 g. on a dry weight basis was weighed into each of the 500-ml. reaction flasks. These 500-ml. flasks previously were washed with a detergent, rinsed well with tap water, soaked in 10 per cent HCl acid overnight, rinsed with tap water, rinsed with distilled water, and air-dried. Each flask was tightly covered with wax paper until the treatments could be applied. Ten ml. of a saturated solution of $Ca(OH)_2$, 1 g. of sucrose, and 2 ml. of a solution containing 2.6870 g. of $Ca(H_2PO_4)_2 \cdot H_2O$ and 4.7800 g. of HCl per 1,000 ml. of redistilled water were added to each flask. To those flask assigned the zineb treatment 5 ml. of zineb solution containing 4.6554 g. of 65 per cent active zineb per 1,000 ml. of redistilled water were added. In the third series, 2 ml. were added of a solution containing 2.8918 grams of NH_4Cl per liter of redistilled water. This was followed by the addition of 2 ml. of a solution containing 3.7848 g. of $ZnCl_2$ per liter of redistilled water and 0.0070 g. of elemental S. This provided Zn, N, and S equivalent to that in the zineb.

The soils were brought to 50-per cent saturation with redistilled water. The assembly of the reaction flasks was completed by connecting with the scrubbers containing 4 per cent H_3BO_3. Duration of the experiment was from April 16, 1962, to May 21, 1962. A companion study using 36 other reaction flasks was identical to that described above except that sucrose was omitted where zineb was added, and sucrose was added at the C content equivalent to that of the zineb in the other flasks.

When the experiments were completed the CO_2 evolved was determined by the titration as shown in Chapter IV. The soil was removed, mixed well and analyzed for extractable Zn, NH_4-N, NO_3-N, SO_4-S, pH, and moisture contents. The CO_2 evolution was the only measurement made of the reaction flasks using either no sucrose or sucrose at the level of C equivalent to the zineb added.

Microbiological experiment 2.--The concentration of zineb was doubled over that used in the above experiment to investigate further the degradation products of zineb and to study the effect on microbial activity, possible interaction of copper, and copper in combination with zineb in the microbial process. The apparatus used in this experiment was the same as used in the first experiment with two modifications. The modifications were that 40 reaction units were used instead of 72, and the H_3BO_3 was located in the absorption train after the carbon dioxide absorbent rather than in a separate experiment. The carbon dioxide absorbent was increased to 0.5 N NaOH. The A2 horizon of the Pomello soil was inoculated with the Arredondo extract as described in the first experiment and used in this experiment.

A completely randomized experimental design with four replications was used. The treatments were as shown in table 2.

The amount of soil per flask and the procedure for applying the treatments were the same as in the first experiment. The concentrations of the zineb, NH_4Cl and $ZnCl_2$ solutions were doubled and the S addition was increased to 0.0140 g. The additional treatments were made by using 5 ml. of a solution of 0.4025 g. of $CuCl_2 \cdot 2H_2O$ per liter of redistilled water and 5 ml. of a nabam solution containing 43.08 ml. of 22 per cent active nabam as stated in Chapter III, per liter of redistilled water which resulted in a final concentration of 200 ppm. or when diluted 1:1 with water was 100 ppm. The addition of sucrose per flask as the energy source was tenfold that used in Experiment 1. After the treatments were applied on July 16, 1962, the moisture content was raised to one-half the water holding capacity of the soil. The CO_2 was collected and titrated, as described earlier in Chapter IV, periodically during the experiment.

The final titration of the collected CO_2 was made on August 25, 1962. The soil was removed from the reaction flask, mixed well, and aliquots were weighted out for the determinations of moisture, pH, NH_4-N, NO_3-N, SO_4-S, and Zn. The preparation and procedure for these analyses were as described earlier in Chapter IV.

Magnesium experiment 1.--The objective of this experiment was to determine if magnesium influenced the availability of zinc derived from zineb or sulfate added to the soil. This experiment was conducted in outdoor lysimeter jars having 5-gallon capacity. These jars were washed with a detergent, rinsed with 25 per cent HCl, rinsed with tap

TABLE 2

Treatments used in a study of the degradation of zineb in Pomello fine sand

Treatment Numbers	Concentration of Materials Added to the Soil						
	Zineb	Nabam	Zn	N	S	Cu	Remarks
	ppm.						
1	0	0	0	0	0	0	Treatments 1 to 9 received 5.37 mg. of $Ca(H_2PO_4)_2 \cdot H_2O$ and 9.5 mg. of KCl. Energy was supplied as 10 g. of sucrose per flask.
2	100	0	0	0	0	0	
3	0	0	24	10	46	0	
4	100	0	24	10	46	0	
5	0	0	24	10	46	5	
6	100	0	0	0	0	5	
7	100	0	24	10	46	5	
8	0	100	0	0	0	0	
9	0	200	24	10	46	0	
10	0	0	0	0	0	0	Soil only

water and then with deionized water. They were filled within an inch of the top with the Arredondo fine sand described in Chapter III that had been screened through a 16-mesh plastic screen. After these jars had been exposed to a heavy rainfall, the soil settled further. Sour orange seedlings which had been grown from seed, Chapter III, in builders sand for two months, were transplanted using 5 per jar. On December 7, 1962, one week after transplanting, an 8-2-8 fertilizer described in Chapter III was applied at the rate of 1,000 pounds per acre and incorporated into the soil. The zinc treatments were applied broadcast immediately after this. The lysimeter area was covered by plastic supported on an inverted V-shaped frame and heated electrically to protect the seedlings from frost damage.

A 2 x 2 factorial randomized block was the experimental design used. It consisted of 2 levels of zinc and 2 levels of Mg with 4 replications of each treatment. The 2 levels of Mg were 0 and 65 pounds per acre while the 2 levels of zinc were 0 and 32 pounds per acre.

The seedlings were harvested on June 30, 1962, and the fresh weights of the entire plant, leaves and roots were recorded. The leaves and roots were prepared and analyzed as described in Chapter IV.

Magnesium experiment 2.--An experiment was conducted to determine if Mg influenced the availability to citrus seedlings of Zn derived either from zinc or $ZnSO_4$. This was a greenhouse investigation using 3-gallon lysimeter jars cleaned as described previously in Chapter V. This experiment was conducted with Arredondo fine sand and with Leon fine sand. These soils had been screened through a 16-mesh plastic screen before being placed in the jars.

On December 2, 1961, sour orange seedlings which were 1-year old and which had been selected by their size and stem diameter were transplanted into the jars. One week later 1,000 pounds per acre of 8-2-8 or the 8-2-8-6 6 fertilizer was applied to the respective treatments and mixed into the soil surface while the zineb treatments were applied to the surface in a suspension.

A randomized block experimental design was used with 2 rates of Mg and 2 materials with 4 replications of each treatment. The 2 rates of Mg were 0 and 65 pounds per acre, while the two materials, zineb and $ZnSO_4$, were applied at one rate which was equivalent to Zn in 32 pounds per acre of the commercial zineb.

After 39 weeks the seedlings were harvested and the fresh weights of the entire plant, leaves, and roots were recorded. The number of new leaves were also noted and they, along with the roots, were prepared and chemically analyzed as described previously in Chapter V.

Copper experiment.--The effects from the application of zineb to a soil which had received a high rate of Cu were investigated by using sour orange seedlings. Three-gallon lysimeter jars were used and these had been cleaned as previously stated in Chapter IV. These jars were filled with Arredondo fine sand that had been screened through a 16-mesh plastic screen. After the soil had been thoroughly wet by rainfall and allowed to dry out a little on the surface, the 6-6-6 fertilizer described in Chapter III was applied at the rate of 1,000 pounds per acre.

A completely randomized experimental design was used with 4 treatments replicated 4 times. The treatments were additions of no Cu,

Cu plus zineb and Cu plus nabam. Copper sulfate was used at the rate of 200 pounds of Cu per acre as a top-dressing applied with the fertilizer and mixed in the soil to a 1-inch depth. The rate of zineb was 32 pounds per acre of the commercial product, Chapter III. The nabam was equivalent to the dithiocarbamate content of the zineb used. Both these treatments were top-dressings to simulate spray residue. After the treatments were applied, the leachate experiment which is described subsequently in Chapter V, was conducted. When approximately 16 l of rainwater had leached through these lysimeter jars, the leachate experiment was concluded.

On September 10, 1961, 10 sour orange seeds, as in Chapter III, were planted in each lysimeter jar having the above treatments. When all of the seed had germinated and were about 3 weeks old, they were thinned to 5 plants per jar and the 8-2-8 and 8-2-8-6-6 fertilizer were applied at the rate of 1,000 pounds per acre. Protection against frost damage was the same as described in the first Mg experiment.

Observations were made of the seedlings during the course of the experiment. They were harvested on June 30, 1962 and the fresh weight of the plants, their leaves and their roots were recorded. The leaves and roots were prepared and chemically analyzed as previously stated in Chapter IV.

Leachate experiment.--A leachate experiment was conducted to determine if zineb, the Zn from zineb, or the organic component of the zineb molecule would leach from the soil, and if high concentrations of Cu affected any such leaching. The equipment, materials, and treatments were described earlier in Chapter V.

The leachate was collected in gallon jugs and analyzed for zineb or the dithiocarbamate as described earlier in Chapter IV. The analysis of Zn from leachates also was made as described before in Chapter IV.

Vertical distribution of applied zinc.--The vertical distribution of Zn from zineb or $ZnSO_4$ was determined by taking soil samples to a depth of 4 inches at 1-inch increments from the Zn-containing treatments of the previous experiment, Chapter V. These soil samples were extracted and analyzed for Zn as described earlier in Chapter V. The concentration of zinc at each depth was used to determine the vertical distribution of the Zn compound applied to the surface of the Arredondo soil.

CHAPTER VI

RESULTS AND DISCUSSION

Effect of Zinab on Rough Lemon Seedlings in the Presence or Absence of a Toxic Concentration of Copper in a Solution Culture

Zinab with high copper.--Growth of the seedlings in this experiment was not uniform. This lack of uniformity could be attributed primarily to individual seedlings showing a wide range of growth response in these solutions. It was observed that under the conditions of careful control of aeration and nutrient supply, the previously starved seedlings did not put out new leaves or new roots at the same time. This resulted in some seedlings growing faster in the same nutrient treatment than did others. This was reflected in the fresh weight of new foliage. The individual values are shown in appendix table 4 and the average value for each treatment in table 3. This pattern of reporting the data is followed throughout in this experiment.

The level of zinab in the solution culture did not significantly affect the fresh weight of new foliage, the increased weight of the entire seedling, the number of new leaves, the green weight of new leaves, the dry weight of new leaves, increase in root volume, and dry weight of roots. Precision of the measurements of each of these effects was limited by the large variability between replicates. Plant symptoms indicative of nutritional disorder were not observed on the leaves except for a slight chlorosis where zinab was not in the solution. However, the roots did show a symptom attributable to copper injury.

TABLE 3

Effects of levels of zineb in solution culture in the presence of 0.5 ppm. of Cu on new leaf growth and chemical composition

Level of Zineb	Leaf Measurement		Leaf Composition							
	Dry Weight	New Leaves	Zn	Cu	Mn	Fe	Ca	Mg	K	P
ppm	g	No per plant	ppm				%			
0	1 5	52	20.0	7.6	22	83	0.81	0.24	1.80	0.24
6.6	2.4	64	21.8	4.9	17	53	0.73	0.22	1.67	0.21
46.6	4.5	98	24.7	3.3	9	63	0.77	0.20	1.50	0.24
86.6	3.0	64	64.4	2.3	14	59	0.82	0.20	1.97	0.26
126.6	3.4	99	88.6	2.5	13	72	0.94	0.24	2.10	0.25
F value*	0.65	1.1	8.05	4.52	1.2	3.7	0.57	0.45	1.34	0.67
S.E.	0.28	9.4	1.54	0.45	1.4	2.7	.04	.01	.09	.01

*F value for significance is 4.25 and 2.77 at the 1 and 5 per cent levels respectively.

TABLE 4

Effects of levels of zineb in solution culture in the presence of 0.5 ppm. of Cu on root volume, weight and chemical composition

Level of Zineb	Root Measurement		Root Composition							
	Dry Weight	Volume Increase	Zn	Cu	Mn	Fe	Ca	Mg	K	P
ppm.	g.	cc.	ppm.				%			
0	1.7	3.4	36	206	50	420	1.11	0.24	1.31	0.24
6.6	2.6	4.7	54	77	25	684	0.77	0.32	1.11	0.25
46.6	3.3	9.2	344	39	22	1047	1.09	0.26	1.46	0.25
86.6	2.5	5.0	765	30	34	1290	1.31	0.22	2.06	0.24
126.6	3.2	9.6	880	22	36	1513	1.41	0.35	2.55	0.15
F value*	0.78	0.72	50	28.7	3.0	8.5	4.24	2.10	4.90	0.62
S.E.	0.32	1.50	24	6.4	2.8	67.8	.03	.01	.06	.05

*F value for significance is 4.25 and 2.77 at the 1 and 5 per cent levels respectively

This Cu-induced injury resulted in stubby roots with swollen tips. Such roots are shown in figure 1 where the almost spherical root tips are scattered throughout the root system which is labelled D. This root abnormality occurred in this experiment and in other experiments where rough lemon seedlings were used. Zineb definitely prevented development of this root injury at levels of 46.6, 86.6, and 126.6 ppm. in the solution. At these levels, the roots were lighter in color and healthier in appearance than at the levels of 0 or 6.6 ppm. of zineb.

Common incidence of the injury occurred where 6.6 ppm. of zineb was applied but the roots had grown about one cm. before this spherical injury developed. Where the zineb was absent the injury was observed even earlier in the root development. Nematodes were not present, as indicated by microscopic examination of the roots. The Cu level of 0.5 ppm. in solution has previously been established as toxic to citrus roots (67) and since this solution was not changed during the course of this experiment, the original Cu concentration must have been inactivated at the higher levels of the zineb application. Otherwise root injury would have resulted.

Examination of the cellular development of the healthy and injured root tips was made microscopically by thin tissue sectioning. Such sections are shown in figure 2. The apical cells of the injured root tip from solution at the above Cu level without zineb were ruptured and appeared to be in a state of disorganization and deterioration. The cells were enlarged along with this disorganization which gave the appearance of knots at the root tip.

Fig. 1 --Roots of rough lemon seedlings showing swollen knot-like tips where C is low incidence without Cu applied, B is severe injury where the Cu level applied was 0.02 ppm. and D is very severe where the Cu level applied was 0.50 ppm.

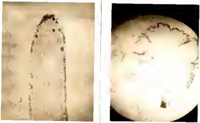

Fig. 2.--Tissue section of the tip of rough lemon root showing normal cellular development in the left photograph and cauliflower-type of cellular abnormalities resulting from excessive Cu in the photograph on the right.

Leaf composition was not affected significantly by the level of zineb in the solution so far as the Ca, P, Mg, K, Mn, or Fe contents of the new leaves was concerned. However, the Cu level decreased significantly at the three highest concentrations of Zineb. The Zn content of the leaves increased significantly at the two highest levels. Calculation of the Zn uptake by these leaves showed an increase in the Zn uptake from 43 up to 447μg from the 0 to the rate of 126.6 ppm of zineb. There was not a corresponding decrease in the copper uptake, although a downward trend was evident. Obviously the increase in Zn content of the leaves and the corresponding decrease in root injury indicate that the Zn from zineb was made nutritionally available to the seedling and without root injury.

Root composition was not affected quite in the same manner as the leaves. Although the Ca content of the roots was depressed at the level of 6.6 ppm of zineb, the P and Mg contents were not altered significantly by the level of zineb in solution. The K content was increased at the highest level of zineb. The Zn content increased greatly at the three highest levels of zineb as shown in table 3. By calculation, the Zn absorption was increased by roots from 53μg without zineb to 140, 1,400, 1,865 and 3,000μg at levels of zineb equivalent to 1, 7, 13 and 19 ppm of Zn respectively. There was a corresponding decrease in Cu content of roots from 206 ppm. to 22 ppm. as the levels of zineb were increased. The Cu absorption by the roots progressively decreased from 320μg to 200, 118, 76, and 70μg with the increasing rates of zineb. This inverse relationship of Zn and Cu, was apparently part of the factors involved in the root injury and the reaction of

zineb in the solution. Copper absorption apparently was inhibited to a large extent by the presence of zineb, and yet the leaf analysis showed that a sufficient amount of Cu was entering the seedlings. However, Fe content of the roots also increased as the rate of zineb was increased and may also have reduced the copper injury.

Zineb without copper applied.--This experiment was conducted at the same time as the previous experiment except that Cu was not applied. The non-uniformity in growth was again noticeable and was attributed to the individual growing characteristics of each seedling. As in the previous experiment, the levels of zineb in the solution culture did not significantly effect the fresh weight of new foliage, the increased weight of the entire seedling, the number of new leaves, the green weight of new leaves, the dry weight of new leaves, the increase in root volume, or the dry weight of roots. The plants showed no visible leaf symptoms of any deficiency. The roots of the checks did have small spherical knots, as shown in figure 1, which were attributable to Cu injury as indicated by analyses of the roots. This Cu probably was introduced with the deionized water. This injury was not noticed on the roots of the treatments receiving zineb which were light in color and appeared to be healthy. As shown above, the Zn content of the leaves was increased with the higher levels of zineb. This increase was from 25 ppm. where no zineb was added to 88 ppm. where 126.6 ppm. of zineb was added. At the same time there was a reduction in the Cu content of the leaf as the Zn content increased. This was evidently due to zineb inactivating the Cu that was introduced by contamination.

TABLE 5

Effect of levels of zineb in solution culture on new leaf growth and chemical composition of leaves

Level of Zineb	Leaf Measurement		Leaf Composition							
	Dry Weight	New Leaves	Zn	Cu	Mn	Fe	Ca	Mg	K	P
ppm	g	No per plant	ppm				%			
0	1.48	49	25.0	13.1	16	66	0.88	0.25	1.94	0.24
6.6	2.41	70	23.3	4.7	12	62	0.75	0.23	2.06	0.27
46.6	4.49	84	50.3	2.8	18	59	0.89	0.20	1.72	0.24
86.6	3.00	64	74.6	2.1	15	57	0.96	0.26	1.96	0.25
126.6	3.39	75	88.3	2.3	14	62	0.90	0.27	1.86	0.34
F value*	2.15	0.8	5.79	2.40	0.3	.1	0.25	0.38	1.30	3.00
S.E.	0.11	6.7	3.40	1.21	1.8	5.9	0.07	0.02	0.16	.01

*F value for significance is 4.25 and 2.77 at the 1 and 5 per cent levels respectively.

TABLE 6

Effect of levels of zineb in solution culture on root volume, weight and chemical composition of root

Level of Zineb	Root Measurement		Root Composition							
	Dry Weight	Volume increase	Zn	Cu	Mn	Fe	Ca	Mg	K	P
ppm	g	cc.	ppm				%			
0	1.48	2.4	13	27	51	247	1.54	0.17	1.88	0.37
6.6	1.81	2.6	106	17	52	479	1.24	0.13	1.22	0.34
46.6	2.55	8.3	452	7	31	747	1.13	0.42	2.08	0.14
86.6	2.44	6.0	703	6	34	1089	1.47	0.21	1.65	0.23
126.6	2.19	5.6	1,153	7	36	900	1.15	0.24	1.59	0.18
F value*	8.11	1.11	101	15.9	1.5	2	1.62	1.37	0.80	4.35
S.E.	0.24	1.06	19	3.3	3.3	89.4	.03	.01	0.08	.01

*F value for significance is 4.25 and 2.77 at the 1 and 5 per cent levels respectively.

There was no significant difference in the Mn, Fe, Ca, Mg, K, or P content of the leaves attributable to the zineb levels applied.

As shown by the root composition, the Zn content of the roots increased significantly as the levels of zineb increased. At the same time the Cu content of the roots was lowered considerably. This differs from the first experiment in that the Cu content was 27 ppm. compared to 206 ppm. Also where the zineb level was 126.6 ppm., the Cu was 7 ppm. compared to 22 ppm. in the first experiment. There was also a trend for the Fe content of the root to increase as the zineb level increased. There were no significant differences in the Mg, Ca, K, and Mn content of the roots with these levels of zineb. The P content of the roots was decreased at the three highest levels of zineb.

Effect of Zineb, Exposed Zineb and Zinc Sulfate on Sour Orange Seedlings in a Solution Culture

The sour orange seedlings in this experiment grew more slowly than the previously described rough lemon seedlings. The data from this experiment, shown in table 7, were assembled from the plants which had initiated new growth. As shown in this table, the growth was not uniform and this resulted in considerable variability in the analyses. This variability was attributed partly to the various ages of the new growth, but was confounded by root injury resulting from a lack of sufficient aeration caused by a temporary failure in the air supply. There was an increase in Zn content of the leaves where $ZnSO_4$ and exposed zineb were supplied and to a lesser extent where zineb was used. This was similar to the results shown in the previous experiments. The Zn content of the leaves from the treatment that received the $ZnSO_4$ was considerably higher

TABLE 7

Effects of zineb, exposed zineb and $ZnSO_4$ at several rates on the new leaf growth and chemical composition of sour orange seedlings grown in solution culture

Zn Supplied	Dry Weight of Leaves	Chemical Composition						
		Zn	Cu	Mn	Fe	Ca	Mg	K
ppm.	g.	ppm.				%		
Check								
0	2.4	11	7.0	39	71	0.80	0.26	1.98
Zineb								
1	1.2	18	6.0	30	68	0.66	.38	1.31
7	2.7	14	6.5	26	64	0.56	.22	1.40
13	1.9	36	4.8	44	56	1.12	.29	1.60
19	.7	44	7.2	34	63	.59	.27	1.72
Exposed zineb								
1	1.6	30	7.4	27	80	0.74	.22	1.24
7	1.1	36	7.2	55	63	1.23	.32	1.88
13	1.6	36	3.1	36	64	1.15	.27	1.54
19	2.2	34	3.7	60	59	0.63	.23	2.01
$ZnSO_4$								
1	2.0	60		31	68	1.15	.26	1.36
7	2.1	288	7.0	44	64	0.77	.22	1.32
13	1.0	292	7.6	36	63	0.90	.33	1.41
19	1.2	258	5.7	29	56	0.96	.32	2.62

than from the 2 zineb sources There was a trend for a reduction in Cu content of the leaves with the highest rates of the exposed zineb treatments. This trend was not evident in the zineb treatment due to the higher Cu value obtained with the highest level of zineb. This high Cu value might be attributed to the small sample size Where the $ZnSO_4$ was used, the Cu content was relatively high except for the highest level This was higher, however, then that obtained for the higher levels of the exposed zineb treatments. There was little change in the Mn content of the leaves due to treatments and these Mn values were unaccountably higher then these in the previous experiment The difference in species probably accounted for this and other changes in the chemical composition resulting from the treatments in these experiments There was essentially no difference in the Fe, Cu, Mg, and K contents of the leaves from the different treatments

Data on the root measurements and analysis are shown in table 9 The Zn content of the roots increased greatly at the highest levels of each material Compared to the zineb treatment, the exposed zineb treatment appeared to affect the Zn content of the roots more readily as indicated by the greater Zn concentration The increase in Zn content of the roots from the $ZnSO_4$ reflected the rates applied more than that from the zineb sources There was not, however, the decided reduction of Cu content of the roots by the increasing levels of zineb as noted in the previous experiment This behavior might have been a species response or it could have been altered by the presence of dead roots which have been reported by Smith and Specht (67) as being an efficient

TABLE 8

Effects of zineb, exposed zineb and $ZnSO_4$ at several rates on the root growth and chemical composition of sour orange seedlings grown in solution culture

Zn Supplied	Dry Weight of Roots	Chemical Composition						
		Zn	Cu	Mn	Fe	Ca	Mg	K
ppm.	g.			ppm.			%	
				Check				
0	4.6	95	12	35	250	0.60	0.31	.46
				Zineb				
1	4.1	97	36	54	1,140	0.70	1.46	1.70
7	5.8	686	20	90	715	1.15	.71	2.70
13	3.1	658	19	78	749	0.49	.42	1.10
19	2.6	532	23	81	1,180	0.85	.52	.86
				Exposed zineb				
1	2.3	325	46	136	986	0.60	.87	2.21
7	3.1	1,770	50	170	1,370	0.89	.56	1.85
13	2.0	1,120	54	134	1,150	0.49	1.26	2.22
19	5.5	1,270	29	58	736	1.07	.36	.82
				$ZnSO_4$				
1	4.8	176	36	54	854	0.94	.48	.74
7	4.2	548	22	92	800	0.48	.45	1.10
13	3.4	1,250	18	116	1,250	0.67	.51	1.36
19	3.8	1,330	23	170	1,440	1.96	.95	1.86

absorbant of Cu. The Mn and Fe contents of the roots were erratic, and increased over the check where the exposed zineb or $ZnSO_4$ was applied. The Ca, Mg, and K contents of the roots showed no treatment effects.

Effect of Exposure to Sunlight on Zineb

Chemical analysis for stability.--Using the method as described in Chapter IV, the recovery of zineb exposed to bright sunlight for 0, 8, 32, 56, and 112 hours was 100.0, 106.5, 110.1, 92.6, and 91.0 per cent, respectively. The precision of this method was not sufficient to justify that there were any real differences from these exposures. Since the method is based upon the evolution of CS_2 by acid hydrolysis upon heating of zineb or certain of the derivatives of the dithiocarbamates, it did not distinguish between the material being analyzed and the degradation products present. When zineb which had not been exposed was mixed with a slurry of fresh plant material, the zineb recovery was reduced to 60 percent. This method, with variations in determining the CS_2 evolved, has been used to establish the residue tolerances on vegetables and fruits for the dithiocarbamate pesticides.

X-ray diffraction analysis for stability.--The zineb placed under direct sunlight for various periods of time gave practically identical x-ray diffraction patterns, as shown in table 9. Obviously any rupture of the zineb crystals was not of sufficient magnitude to destroy or shatter the crystal since both the spacings and their relative intensities remained unaltered by these exposures. The character of these patterns indicated that the zineb crystals were well ordered and highly crystalline. Any surface deterioration of the crystals by the action of the sunlight was not serious enough to alter the structure of the zineb materially

TABLE 9

Spacings and relative intensities of X-ray diffraction patterns of zineb after different exposures to sunlight, chloroform leaching and of the ash heated to 400°C for 4 hours

Zineb Treatment	Relative Peak Intensities 100	70	65	32	30	Other Weaker Lines
	Spacing, A					
Unexposed	6.91	3.45	4.82	3.03	4.58	3.63, 2.12, 2.80, 3.30
Exposed 8 hr	6.91	4.82	3.45	4.50	3.03	3.63, 2.72, 2.80, 3.30
Exposed 32 hr.	6.91	4.82	3.45	3.03	4.50	3.63, 2.12, 2.80, 3.30
Exposed 56 hr.	6.91	4.82	3.45	4.50	3.03	3.63, 2.80, 2.12, 3.30
Exposed 112 hr.	6.90	4.82	3.45	4.50	3.03	3.63, 2.80, 2.12, 3.30
Chloroform extn.	6.90	4.82	3.45	3.03	4.50	3.63, 2.80, 2.12, 3.30
Ashed	**100**	**90**	**40**	**36**	**33**	**Others**
	Spacing, A					
400°C for 4 hours	2.83	2.49	1.96 1.63	3.13	2.61	3.31, 3.26, 4.69, 2.94 2.00, 2.65, 2.33, 1.87

Chemical degradation of the crystals also must have been essentially negligible since the relative intensities remained constant and no new spacings were formed. Similarly the extraction with chloroform did not dissolve the zineb. This was confirmed by analysis of this product which showed 16.0 per cent Zn which is the same as formed in dry zineb containing the carrier. Search of the literature failed to reveal a previously published x-ray diffraction pattern for zineb.

The inert carrier of this commercial zineb was amorphous since none of the spacings could be attributed to those of clay minerals, likely to be used as the carrier. The spacing at 4.82Å was not gibbsite since the spacings at 4.37Å and 2.39Å were absent. Similarly, when the sample was ashed at 400°C none of the spacings, except the weak one at 3.30Å, were alike those in the zineb. Crystalline clays have sharp diffraction patterns after such a treatment. The ash contained a mixture of Zn compounds which probably included the oxide, carbonate, sulfide and silicate crystals. None of these spacings were attributable to a known clay mineral. Since Al was very low in the ash the carrier was probably a silicate such as diatomaceous earth. This carrier might have protected the zineb from degradation by sunlight.

<u>Biological analysis for stability</u> --In this experiment, the leaves of the rough lemon seedlings appeared normal except for a slight chlorosis where $ZnSO_4$ was used. The data are shown in table 10. The Zn content of the leaves was increased by the zineb and $ZnSO_4$ treatments. This increase in Zn content of the leaves was similar for zineb at all 4 exposures but lower than the treatment with $ZnSO_4$. The Cu content of leaves from the check treatment and that with $ZnSO_4$ was much higher than the Cu content of the leaves receiving the exposed zineb. This

indicated that the presence of Zn alone was not the cause for the lowering of the Cu content of the leaves by this zineb. There was not a significant difference between the Cu content of the leaves grown in solution with the differently exposed zineb. In this experiment the Ca content of the leaves was significantly affected by the zineb treatments.

The roots from the above plants exhibited some of the knots at the tips noted previously where Cu was presumably toxic. However, this condition existed only where Zn was not supplied or $ZnSO_4$ was added. As before, the knots were not present where the zineb was present in the solution culture. The data are given in table 11. The $ZnSO_4$ treatment produced roots which were the highest in Zn and P content. The exposed zinebs were alike in the large increase in Zn content of these roots over the check and the corresponding drop in the Cu content. The exposed zinebs did significantly change the root volume. Since the other elements were not altered by the zineb the Zn:Cu ratio was probably involved in this benefit although the exact nature of this reaction was not obvious from this experiment.

When the Zn supply was increased from 7 to 13 ppm. the leaves again appeared to be healthy and normal in growth. The data for the leaves are shown in table 12. There were fewer leaves when the $ZnSO_4$ was present in the solution than for the other treatments. The Zn content of these leaves showed a wide range within treatment and differences between treatments were not significant. The Cu content of the leaves was decreased where the zineb was present in the solution culture. The differences in the K and P content were significant but Mn, Fe, Ca and Mg were not significantly changed by the Zn sources.

TABLE 10

Effects of exposed zineb and $ZnSO_4$ applied at 7 parts per million of Zn on the new leaf growth and leaf composition of rough lemon seedlings grown in the solution culture

Material	Sun. Exp.	Leaf Measurements		Leaf Composition							
		Dry Weight	New Leaves	Zn	Cu	Mn	Fe	Ca	Mg	K	P
	hr	g	No per plant	ppm				%			
Check		1.8	69	32	13.2	13	52	0.86	0.23	2.27	0.23
$ZnSO_4$		2.1	54	154	8.8	22	56	0.83	0.24	1.74	0.23
Zineb	8	5.3	68	62	2.2	12	45	0.89	0.23	1.72	0.23
Zineb	32	4.3	89	73	3.9	16	64	1.04	0.29	2.00	0.28
Zineb	56	4.2	70	48	2.6	14	63	0.93	0.24	1.78	0.24
Zineb	112	3.9	66	71	3.3	15	61	0.69	0.23	2.06	0.23
F value*		2.47	2.17	3.61	10.4	0.92	0.88	14.05	0.55	0.83	0.75
S.E.		.04	3.00	28.8	0.56	1.32	3.23	.01	0.14	.09	.03

*F value is 4.25 and 2.77 for significance at the 1 per cent and 5 per cent levels respectively

TABLE 11

Effects of exposed zineb and $ZnSO_4$ applied at 7 parts per million of Zn on the root growth and root composition of rough lemon seedlings grown in the solution culture

Material	Sun. Exp	Root Measurements		Root Composition							
		Dry Weight	Volume Increase	Zn	Cu	Mn	Fe	Ca	Mg	K	P
	hr	g	cc.	ppm.				%			
Check		1.6	2.1	12	22	18	388	1.29	0.25	1.09	0.28
$ZnSO_4$		1.9	3.1	1041	20	22	633	1.53	0.33	1.34	0.48
Zineb	8	3.2	9.6	433	5	32	712	1.22	0.38	1.83	0.23
Zineb	32	2.6	6.1	542	7	53	796	1.27	0.35	1.59	0.20
Zineb	56	3.2	7.1	427	6	33	914	1.19	0.34	1.50	0.27
Zineb	112	2.4	9.1	566	8	37	662	1.12	0.33	1.70	0.26
F value*		1.77	7.33	30.8	12.6	1.28	1.30	1.64	0.84	1.57	6.23
S.E.		0.20	0.65	23.9	0.94	0.36	62.8	.02	.01	.05	.02

*F value is 4.25 and 2.77 for significance at the 1 per cent and 5 per cent levels respectively

From the root composition, as shown in table 13, the Zn and Cu contents of the roots were alike those found at the lower Zn level. The Cu content of the check and of the treatment with $ZnSO_4$ was higher than the values obtained where differently exposed zinebs were used. However, the degree of sunlight exposure of the zineb was not apparently a factor in the Cu and Zn levels accumulated by the roots from zineb. The Mn, K, and Mg contents of the roots were significantly higher than those of the check or $ZnSO_4$ treatments. The Fe, Ca, and P contents of the roots were not significantly different between treatments.

The stability of the zineb was not likely to have been changed greatly by the exposure to sunlight since the response by the seedlings and the chemical composition of both leaves and roots were alike for exposures of 8 up to 112 hours. A change in the Zn availability was not encountered with these exposures.

Zineb in the Soil

Microbiological experiment I

Soil analysis.--The data for the first microbiological study are shown in table 14. Significant amounts of Zn were extracted from the treatments where zineb and the inorganic Zn source were used. According to these results, approximately 4 ppm. of the Zn applied as an inorganic source were fixed by the Pomello soil in the incubation process. Assuming that the same amount was fixed in the treatment where zineb was used, the 5 ppm. of Zn extracted plus the 4 ppm. fixed indicated that probably 9 of the 12 ppm of Zn applied as zineb were released in the incubation process. The NH_4-N extracted was significantly more in the treatments where zineb was applied than where NH_4-N was added or in the control. Approximately 40 per cent of the N applied was recovered

TABLE 12

Effects of exposed zineb and $ZnSO_4$ applied at 13 parts per million of Zn on the new leaf growth and leaf composition of rough lemon seedlings grown in the solution culture

Material	Sun. Exp.	Leaf Measurements: Dry Weight	Leaf Measurements: New Leaves	Leaf Composition: Zn	Cu	Mn	Fe	Ca	Mg	K	P
	hr.	g.	No per plant	ppm.				%			
Check		1.9	64	17	7.6	13	46	0.86	0.29	2.05	0.25
$ZnSO_4$		0.8	31	135	11.0	25	69	1.01	0.33	1.87	0.18
Zineb	8	2.9	58	60	3.0	20	71	0.89	0.27	1.96	0.26
Zineb	32	4.8	80	59	3.0	9	53	1.08	0.25	1.94	0.26
Zineb	56	2.9	70	53	2.7	15	44	0.94	0.22	2.11	0.24
Zineb	112	3.7	73	67	1.9	16	36	0.96	0.23	1.70	0.26
F value*		2.74	3.19	1.86	19.56	1.14	1.78	0.43	2.12	3.93	2.83
S.E.		0.34	3.92	1.15	0.33	1.53	4.10	.05	.01	.03	.01

*F value is 4.25 and 2.77 for significance at the 1 per cent and 5 per cent levels respectively.

TABLE 13

Effects of exposed zineb and $ZnSO_4$ applied at 13 parts per million of Zn on the root growth and root composition of rough lemon seedlings grown in the solution culture

Material	Sun Exp.	Root Measurements: Dry Weight	Root Measurements: Volume increase	Root Composition: Zn	Cu	Mn	Fe	Ca	Mg	K	P
	hr.	g.	cc.	ppm.				%			
Check		1.8	2.3	14	18	18	362	1.64	0.20	1.12	0.24
$ZnSO_4$		1.3	0.6	1007	22	12	627	1.63	0.27	1.18	0.36
Zineb	8	2.9	5.7	588	6	40	964	1.22	0.39	1.66	0.25
Zineb	32	3.8	11.0	546	5	28	958	1.48	0.46	2.20	0.29
Zineb	56	2.4	3.4	554	5	28	894	1.18	0.31	1.50	0.23
Zineb	112	3.2	6.0	588	4	26	668	1.24	0.34	1.66	0.27
F value*		2.40	1.87	8.50	11.0	2.79	1.61	1.31	1.45	3.01	.08
S.E.		0.23	1.08	44.2	0.10	2.44	76.6	.04	.02	.05	.02

*F value is 4.25 and 2.77 for significance at the 1 per cent and 5 per cent levels respectively.

TABLE 14

Microbiological study of degradation of zineb by comparison with the equivalent inorganic salt addition and the control using Pomello fine sand where A is not inoculated, B inoculated with extract from Leon fine sand, and C inoculated with extract from Arredondo fine sand

Treatment	Soil	Zn	NH_4-N	NO_3-N	SO_4-S	pH	
		ppm				H_2O	1N KCl
Zineb	A	5.4	1.6	0.3	0.9	5.35	4.65
	B	5.0	2.3	0.2	0.7	5.41	4.64
	C	5.2	2.0	0.3	1.4	5.45	4.70
Inorganic Zn, N and S equivalent to zineb	A	8.6	0.5	0.7	1.2	4.55	4.12
	B	8.5	0.4	0.4	0.6	4.54	4.12
	C	8.3	0.3	0.4	0.7	4.56	4.14
Control	A	0.2	0.1	0.6	0.2	5.46	4.65
	B	0.2	0.1	0.5	0.8	5.51	4.65
	C	0.2	0.1	0.3	0.9	5.51	4.65
F value*		156	5.41	1.07	0.94	273	195
S. E.		0.10	0.10		0.12	.05	.01

*F value for significance are 3.26 and 2.30 at the 1 and 5 per cent levels respectively.

as NH_4-N. The NH_4-N recovered from the treatment where an inorganic source was applied was higher than the control, but less than 10 per cent of that applied. The NO_3-N and SO_4-S were not significant with approximately equivalent amounts being extracted from all treatments. The low quantities of S and N recovered may have been due to the organisms needing these elements for their own growth and reproduction. The pH in water of the zineb treatments was slightly lower than the control. The treatments containing the inorganic source of S had a pH in water of approximately 1 unit lower than the other treatments. In N KCl the pH of the control and the zineb treatment were the same. The pH of the treatment where the inorganic source of S was applied was approximately a 0.5 unit lower than the control. This lowering of the pH where the inorganic S was applied was the result of the oxidation of the S forming SO_4-S and also to a lesser extent due to the acid formed when NH_4-N has oxidized to NO_3-N.

The Pomello soil reacted similarly whether it was not inoculated or the other 2 soil inoculants were added. Since the energy source was only 1 gm. of sucrose per 300 g. of soil the microbial activity was limited in this 30-day trial. Zineb at this concentration of 50 ppm. was apparently partially decomposed as shown by the Zn and NH_4-N extracted by the 10 per cent NaCl.

The CO_2 evolved, shown in appendix table 8, was alike for soil treatments and variations.

Microbiological experiment 2

Soil analysis.--Results of the soil extraction after completing the second microbiological study are shown in table 15. Approximately

TABLE 15

Results of second microbiological study on the degradation of zineb with comparisons using equivalent inorganic salts or nabam and including a Cu variable

Treatment[a]	Zn	NH_4-N	NO_3-N	SO_4-S	Final pH		Initial pH	
	ppm.				H_2O	$\frac{N}{}$ KCl	H_2O	$\frac{N}{}$ KCl
1	0.2	6.0	0.3	0.2	2.77	2.65	5.65	4.60
2	7.5	10.9	0.4	0.3	2.69	2.58	5.70	4.80
3	18.0	6.8	0.7	1.4	3.10	3.01	5.30	4.80
4	25.5	6.2	1.1	6.4	3.24	3.12	5.30	4.80
5	21.8	6.8	0.8	0.6	3.42	3.34	5.00	4.55
6	6.9	7.5	0.8	0.8	2.88	2.78	5.45	4.60
7	23.6	6.0	0.8	7.3	3.42	3.32	5.35	4.90
8	0.2	7.4	2.2	6.0	3.94	3.60	6.55	5.35
9	13.8	7.0	0.6	11.4	3.75	3.58	6.00	5.40
10	0.1	1.6	0.5	0.1	5.12	4.38	5.85	4.60
F value[b]	40.3	2.62	2.56	16.3	24.8	19.79		
S.E.	0.500	0.444	0.334	0.314	0.456	.064		

[a]See table 2 for treatments.

[b]F values for significance are 3.06 and 2.21 at the 1 and 5 per cent levels respectively.

18 of the 24 ppm. of Zn applied in the inorganic form were recovered in the extraction. However, approximately one-third of the Zn was extracted from the zineb as indicated in treatment 2. Proportionally, one-half of the Zn from zineb was released, or calculated on the basis of the amount that was tied up in the inorganic treatment, 50 per cent of the Zn in the zineb was released. In treatment 4 where zineb and the inorganic Zn were applied, the Zn extracted approached the total extracted from zineb in treatment 2 and the inorganic source in treatment 3. When Cu was added with the inorganic salts in treatment 5, the amount of Zn extracted was more than that with inorganic salts alone which is treatment 3. This was probably due to Cu occupying the exchange sites formed during incubation and releasing the Zn where it could be extracted. In treatment 6 where Cu and zineb were applied, there was a large reduction in Zn extracted. As will be shown later, the microbial activity of this treatment was lowered. This might indicate that the degradation process was altered, or slowed down, whereby the Zn was not extractable. This inhibition was not observed in treatment 7 where zineb, inorganic Zn, and Cu were applied and gave extractable Zn as in treatment 4 where only inorganic Zn and zineb were applied. Nabam at 100 ppm. in treatment 8 was very low in extractable Zn since none was intentionally added. However, in treatment 9 where 200 ppm. of nabam were added along with the inorganic materials and Cu, the Zn extracted was considerably less than treatment 5 where only the inorganic materials and Cu were applied. This was probably due to the formation of zineb from the inorganic Zn and nabam, which rendered part of the Zn not extractable.

The significant difference between treatments for extracted NH_4-N shown in table 15 was for zineb alone, in treatment 2. Proportionally the same amount of NH_4-N was extracted in the 2 microbiological experiments. The significant difference between nitrate extractions was attributed mainly to the N released from nabam which was likely to be unstable. In treatment 9 where the inorganic sources plus Cu were applied along with zineb, the NO_3-N was only slightly reduced. In treatment 4 where zineb and the inorganic sources were used in combination, the additive effects of treatment 2 and 3 probably accounted for the slight increase in NO_3-N extracted.

The significant differences between extractable SO_4-S, table 15, was attributable to a number of treatments. The most predominant of these was treatment 9 where 200 ppm. of nabam were used. This was approximately twice the amount obtained in treatment 8 where only 100 ppm. of nabam were used. Where the inorganic sources were used with zineb, treatments 4 and 7, there were about 6 ppm. of SO_4-S compared to the 46 ppm. calculated for complete degradation of the zineb.

The final pH values of all treatments, shown in table 15, except treatment 10 which contains soil only, were much lower than the initial pH at the beginning of the experiment. The high acidities in both water and N KCl were in treatments 1, 2, and 6, which included the check, zineb, and zineb plus Cu, respectively. The main source of the acidity arose from microbial by-products since in this experiment 10 g of sucrose were added per 300 g. of soil which undoubtedly resulted in a much higher microbiological activity than in the previous trial discussed in the above

CO_2 evolution.--The microbiological activities expressed as mg. of CO_2-C evolved are shown as the average amount found for each interval in table 16, as the cumulative amounts in the curves shown in fig 3 and 4, and as the average rate per day for each interval in fig. 5. From table 16, the treatments were very significantly different in their effects on the CO_2 evolution. The comparison between treatments 1 and 2 gives the effect of zineb addition on the sucrose decomposition. The N, S, and Zn equivalent to the 100 ppm. of zineb is the control, which is treatment 3, which can be used to compare the effect of zineb addition to the system in treatment 4. Treatments 5, 6, and 7 are for the evaluation of the effect of 5 ppm. of Cu in the system where the control is treatment 5, 6 is with zineb, and 7 is zineb plus the inorganic equivalents. Treatment 8 is 100 ppm of nabam and its effect is comparable with treatment 2 which is the same rate of zineb, or with treatment 9, 200 ppm. of nabam plus zineb and Cu.

The addition of zineb reduced the total CO_2-C produced during the length of the experiment by 31 per cent from the control, fig 3. The rate of CO_2-C production which was determined for intervals, indicated that the rate where zineb was applied was always lower than the control, fig. 5. With the inorganic source there was a stimulation at the beginning of the experiment but the CO_2-C production rate was rapidly lowered during the next interval and the total CO_2-C produced was 16 per cent lower than the control. When the inorganic source and zineb were combined, as in treatment 4, there was a reduction in total CO_2-C produced of 25 per cent. The combined effects of treatments 2 and 3 are reflected in this treatment by a lag during the first 4 days followed by an increase

TABLE 16

CO_2 evolution from Pomello fine sand at different intervals in the second microbiological study on the degradation of zineb with comparisons using equivalent inorganic salts, or nabam, and including a Cu variable

Treatment*	Days Elapsed from Initiation of Experiment							
	4	10	17	24	31	34	38	45
	CO_2-C evolved in the interval, mg.							
1	10	13	43	37	43	20	40	42
2	4	12	32	25	21	18	32	28
3	32	22	26	23	28	23	24	28
4	7	31	28	22	26	18	24	28
5	30	21	21	14	24	21	20	18
6	5	6	13	14	21	26	23	23
7	21	27	24	17	26	19	21	26
8	1	3	10	11	14	23	16	16
9	1	8	18	18	21	24	23	22
10	1	2	3	2	2	2	3	3
F value**	206.60	38.44	15.23	10.38	7.97	0.31	6.77	4.14
S.E.	.28	.53	.86	.80	.94	1.95	.91	1.43

*See table 2 for the treatments.

**F values were calculated omitting treatment 10 and values of 3.26 and 2.45 or above were required for significance at the 1 and 5 per cent levels respectively.

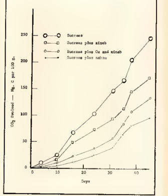

Fig. 3.--Curves of cumulative CO_2 evolved from Pomello fine sand inoculated with an extract of Arredondo fine sand and the treatments are those listed in table 2.

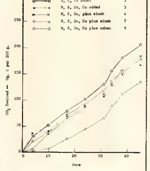

Fig. 4.--Curves of cumulative CO_2 evolved from Pomello fine sand inoculated with an extract of Arredondo fine sand where inorganic salts equivalent to that in the zineb were added and the treatments are those listed in table 2.

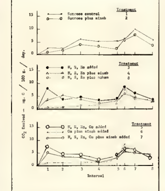

Fig. 5.--CO_2 evolution plotted as the daily average for each interval of measurement where the treatments are those given in table 2 and the media are Pomello fine sand, inoculated with an extract of Arredondo fine sand, and with 3.3 g. of sucrose present.

in rate of CO_2-C production. The presence of zineb retarded the stimulating effect of the inorganic source.

In treatment 5 where Cu was added to the inorganic source, there was the same stimulation as found in treatment 3 which contained only the inorganic source, but during the 17- to 24-day interval the CO_2-C production was reduced and resulted in a total CO_2-C production which was 32 per cent lower than the control. This indicated that Cu had the effect of lowering the microbial activity. This was again reflected in treatment 6 where zineb and Cu were used in combination. The total CO_2-C produced was lower by 46 per cent than the control, which is probably a combination of these two materials inhibiting different microbial populations. The presence of Cu did not reduce the microbial inhibition by zineb, nor did the presence of zineb reduce the antagonistic effect of Cu. In treatment 7, which was additions of zineb and Cu, it was noted that the CO_2-C production was very similar to treatment 4 which contained only the inorganic source and zineb. In this case, however, the inhibition during the first 4 days, prominent in treatments 2, 4, and 6 which also contained zineb, was lessened and the CO_2-C production for that period was increased to twice that of the control. Although this increase was only 50 per cent of the stimulation produced from the inorganic source or the inorganic source in combination with Cu, it showed, in comparison with treatment 4, that the presence of Cu reduced the initial fungicidal activity of zineb.

From the results obtained with treatment 8, it was shown that nabam had the greatest effect upon the microbial activity. In comparison, treatment 9 contained twice as much nabam as treatment 8 but was less

effective due to the addition of the inorganic N, S and Zn with the addition of Cu. Treatment 13 was a treatment containing only soil and water with none of the materials or energy source added.

The relatively high rate of zineb and maneb at the energy level provided by the sucrose showed that while these dithiocarbamates did effect the microbial activity, after about 3 weeks the inhibition was largely overcome. Either a tolerance to these materials was developed by the microbial population or the degradation process yielded products relatively non-toxic to these organisms. Maneb was more toxic than zineb under the conditions of this experiment.

Magnesium experiment I

Differences in treatments could not be distinguished by a visual comparison of the growing seedlings. This uniformity was also reflected in the dry weight of the leaves as shown in table 17. From this table it was noted that there was no significant difference between the Ca, P, Mg, K, Zn, Cu, Mn, and Fe contents of the leaves. Lack of differences between Mg treatments was probably due to the past history of the soil or to the inherent nature of this soil to be sufficient in Mg for this experiment. No differences were noted due to the zineb treatment. This might be due to a lack of contact of the zineb with a sufficient portion of the root system. It will be shown later in another experiment that the zineb, which was applied to the surface, remained near or at the surface.

Although the Mg content of the seedlings was not significant, there appeared to be a trend for a better root system when Mg was

applied, as shown in appendix table 11. According to the data in table 18 the Ca, P, K, Cu, Mn, and Fe contents of the roots were not significant.

Magnesium experiment 2

Differences due to treatments could not be noted from observations of the growing seedlings on either soil type. The Mg addition did not affect the Mg content of the leaves nor did the addition of zineb increase the Zn content of the leaves. No interaction between Zn and Mg was evident. The Ca, P, Mg, K, Zn, Cu, Mn, and Fe contents of the leaves was not significant among treatments. The data are given in table 19.

The roots appeared light in color but no noticeable difference between treatments could be seen. There was no significant difference between the Ca, P, Mg, K, Zn, Cu, Mn, and Fe contents of the roots. As in the leaves, the additions of Mg or zineb did not raise the content of Mg or Zn in the roots. The data are shown in table 20.

Copper experiment

When these seedlings were about 3 weeks old, definite leaf patterns of minor element deficiencies could be seen. The predominant pattern appeared to be Zn, but Mn was involved also. These leaf patterns could not be associated with any treatment and were due to the high pH from a previous application of limestone to the soil before it was collected. After the application of an 8-2-8 fertilizer containing Mg and Mn as described in Chapter III, the seedlings outgrew these deficiency symptoms.

At the time of harvest the treatments could not be distinguished by visual observations. The application of 200 pounds per acre of Cu

TABLE 17

Effect of Mg addition on the growth and chemical composition of leaves of sour orange seedlings grown with and without zineb in unlimed Arredondo fine sand

Treatment	Rate	Dry Weight of New Leaves	Zn	Cu	Mn	Fe	Ca	P	Mg	K
	lb./A	g.	ppm.				%			
Check		14.1	9.2	3.4	21	58	2.11	0.17	0.25	1.03
Zineb	32	15.8	9.0	3.0	25	58	2.06	0.17	0.26	1.04
$MgSO_4$	65	16.2	9.3	3.2	19	40	2.21	0.17	0.26	0.93
Mg plus Zineb as above		16.6	10.7	3.0	21	46	2.07	0.17	0.23	0.93
F value*		2.38	NS	NS	NS	NS	NS	NS	NS	NS
S.E.		0.364								

*F values were not significant.

TABLE 18

Effect of Mg addition on the growth and chemical composition of roots of sour orange seedlings grown with and without zineb in unlimed Arredondo fine sand

Treatment	Rate	Dry Weight of Roots	Cu	Mn	Fe	Ca	P	Mg	K
	lb./A	g.	ppm.			%			
Check		13.3	7.0	32	1,572	0.80	0.19	0.30	1.50
Zineb	32	13.3	6.8	33	1,508	0.65	0.18	0.30	1.44
$MgSO_4$	65	15.2	6.5	34	1,575	0.61	0.18	0.30	1.86
Mg plus zineb as above		14.2	6.6	32	1,395	0.80	0.16	0.27	1.65
F value*		NS	NS	NS	NS	NS	NS	NS	NS

*F values were not significant.

TABLE 15

Effect of Zn from two sources and Mg addition to two soils on the growth and leaf composition of sour orange seedlings

Treatment No.	Material	Rate	Dry Weight of New Leaves	Zn	Cu	Mn	Fe	Ca	P	Mg	K
		lb./A	g.	ppm.				%			
				Arredondo fine sand							
1.	Zineb	32	13.6	14	4.4	8.4	33	3.42	0.19	0.40	0.99
2.	Zineb plus $MgSO_4$	32 65	13.7	12	3.8	9.7	46	3.40	0.16	0.35	0.91
3.	$ZnSO_4$	14	13.6	20	4.7	8.3	21	3.30	0.16	0.36	1.06
4.	$ZnSO_4$ plus $MgSO_4$	14 65	13.6	14	4.7	8.8	46	3.06	0.18	0.34	0.96
F value*			NS	NS	3.98	NS	NS	NS	NS	NS	NS
				Leon fine sand							
1.	Zineb	32	10.7	15	6.5	6.7	40	2.30	0.18	0.47	1.07
2.	Zineb plus $MgSO_4$	32 65	11.0	25	7.2	6.8	37	2.47	0.19	0.42	1.06
3.	$ZnSO_4$	14	10.7	14	7.1	7.1	40	2.08	0.17	0.44	1.21
4.	$ZnSO_4$ plus $MgSO_4$	65	11.8	15	8.0	6.1	37	2.16	0.19	0.48	1.13
F value*			NS	NS	2.05	NS	NS	4.92	NS	NS	NS

*F value for significance is 9.78 at the 1 per cent level and 4.76 at the 5 per cent level.

TABLE 20

Effect of Zn from two sources and Mg addition to two soils on the root growth and root composition of sour orange seedlings

Treatment No.	Treatment Material	Rate	Dry Weight of Roots	Root Composition Zn	Cu	Mn	Fe	Ca	P	Mg	K
		lb /A	g	ppm				%			
Arredondo fine sand											
1	Zineb	32	9.4	55	7.6	37	1,920	0.75	0.17	0.78	1.11
2	Zineb	32									
	$MgSO_4$	65	11.0	54	11.3	52	2,520	0.68	0.21	1.26	1.14
3	$ZnSO_4$	14	10.1	27	7.5	34	1,770	0.59	0.15	0.84	0.95
4	$ZnSO_4$	14									
	$MgSO_4$	65	11.9	34	9.9	38	2,000	0.76	0.17	0.93	1.01
F value*			NS	NS	NS	NS	NS	NS	NS	NS	NS
Leon fine sand											
1	Zineb	32	10.9	112	17.3	28	261	1.19	0.11	0.99	1.13
2	Zineb	32									
	$MgSO_4$	65	9.1	76	16.1	20	184	1.35	0.12	1.07	.93
3	$ZnSO_4$	14	10.0	81	16.8	19	187	1.12	0.12	1.16	1.27
4	$ZnSO_4$	14									
	$MgSO_4$	65	10.0	61	15.8	26	223	1.43	0.16	1.21	1.03
F value*			NS	NS	NS	NS	NS	NS	NS	NS	NS

*F value for significance is 9.78 at the 1 per cent level and 4.76 at the 5 per cent level

did not increase the Cu content of the leaves as shown in table 21. The high soil pH of 6.95 and organic content of the soil rendered this Cu unavailable to the seedlings. There was no increase in the Zn content of the leaves where zineb was applied. As stated previously, this was probably due to a lack of contact of zineb with a sufficient portion of the roots. The K content of the leaves was significant in this experiment, but there was no significant difference in the Ca, P, Mg, Cu, Mn, and Fe contents of the leaves. There appeared to be a trend for a reduction in Mn where Cu alone was applied but this was not significant.

There was a large degree of non-uniformity in the root growth as noted in table 22. The Cu content of the roots was not increased by the additions of zineb. The Ca, P, Mg, K, Mn, and Fe contents of the roots were not significant.

Leachate experiment

The leachates obtained from the treatments were darkened with soluble organic matter from the soil. The presence of this organic colloid in the leachate was brought about by the alkaline condition produced from the heavy liming of this soil. This was reflected in the high pH of the leachate as shown in appendix table 14. The Zn and Cu contents of the leachates varied and no differences could be attributed to treatments. Analysis for the presence of zineb in the leachate by the method of Clarke et al. (9) indicated that none was present.

Infrared analysis of the leachate that had been taken to dryness at 107°C. showed tall narrow peaks at 6.15 and 7.25μ and a wide peak

TABLE 21

Effect of Cu addition with and without zineb or nabam on the new growth and leaf composition of sour orange seedlings grown on limed Arredondo fine sand

Treatment			Dry Wt	Leaf Composition							
Cu	Zineb	Nabam	New Leaves	Zn	Cu	Mn	Fe	Ca	P	Mg	K
lb/A.			g	ppm				%			
0	0	0	16.7	16.3	12.4	21	36	2.39	0.20	0.27	0.86
200	0	0	19.4	15.8	12.5	20	46	2.46	0.18	0.28	0.83
200	32	0	17.0	15.3	13.0	25	45	2.44	0.18	0.28	0.81
200	0	100	21.1	14.5	14.5	24	40	2.50	0.18	0.26	0.71
F value*			2.71	NS	NS	NS	NS	NS	NS	NS	4.37
S.E.											.05

*F value for significance is 5.41 at the 1 per cent level and 3.26 at the 5 per cent level

TABLE 22

Effect of Cu addition with and without zineb or nabam on the root growth and root composition of sour orange seedlings grown on limed Arredondo fine sand

Treatment			Dry Wt	Root Composition							
Cu	Zineb	Nabam	Roots	Zn	Cu	Mn	Fe	Ca	P	Mg	K
lb/A.			g	ppm				%			
0	0	0	18.3	57	41	54	480	0.92	0.16	0.36	0.66
200	0	0	19.5	59	54	61	548	0.90	0.13	0.40	0.72
200	32	0	18.2	59	55	58	551	0.81	0.16	0.36	0.84
200	0	100	21.6	50	55	52	558	0.87	0.17	0.36	0.69
F value*			NS	NS	NS	NS	NS	NS	NS	NS	NS

*F values not significant.

TABLE 23

Analysis of Zn and Cu in leachates from limed Arredondo fine sand with and without copper alone or with zineb or nabam treatments

Leachate	Treatment			
	Copper Level			
	Check	200 lb /A		
		Dithiocarbamate Source		
		None	Zineb	Nabam
			32 lb /A	100 lb /A
Zn content, mg $\times 10^{-5}$				
1	3.8	2.8	2.5	2.2
2	1.4	2.2	1.1	1.2
3	3.5	3.0	3.0	2.9
4	3.2	2.4	2.4	2.6
5	1.5	1.3	1.4	2.9
Total Zn Leached, mg				
Combined	398	284	303	350
Cu content, mg $\times 10^{-5}$				
1	11.5	10.1	9.7	12.4
2	13.8	13.1	11.8	12.7
3	11.7	13.8	11.4	12.9
4	11.8	12.1	11.6	12.3
5	7.2	6.8	7.8	7.0
Total Cu Leached, mg				
Combined	1,600	1,460	1,530	1,640

from 8.5 to 9.1μ . Much smaller peaks were noted at 4.15, 9.95, and 11.90μ . These peaks were the same for samples taken from each of the treatments.

Vertical distribution of applied zinc

The average values presented in table 24 were obtained by analyzing 1-inch increments of soil to a depth of 4 inches, 30 weeks after the treatments had been applied. The treatments were 14.8 ppm of Zn applied as zineb or $ZnSO_4$. The values from the increments with the exception of the 1-inch increment are approximately the same. This indicates that the movement of zineb or Zn from these sources in a downward direction is very slow, if at all. It would appear that a surface application of these materials could not efficiently be used unless better contact was made with the root system.

TABLE 24

The vertical distribution of Zn from zineb and $ZnSO_4$ to a depth of 4 inches, 30 weeks after application

Soil	Zineb*				$ZnSO_4$*			
	Increment of Soil Sample, in.							
	1	2	3	4	1	2	3	4
Arredondo	16.2	5.6	3.0	3.0	12.9	3.6	3.2	2.4
Leon	15.7	3.3	2.7	3.0	12.9	3.6	3.2	2.7

*Surface application at the rate of 14.8 ppm of Zn in the top inch of soil.

CHAPTER VII

GENERAL DISCUSSION AND CONCLUSIONS

Certain properties of zineb in the experiments with citrus seedlings were found that should have a bearing on the use of this pesticide in citrus and perhaps other crops. The most important finding was that the zineb, both in solution culture and in soil, was not toxic to the citrus seedlings. Secondly, the intake of Ca, Mg, K, P, Mn, and Fe by these seedlings was not altered by the presence of zineb or nabam in the nutrient or soil media. Thirdly, the root systems and the above elements in the chemical composition were not significantly changed where contact with zineb was made. Evidence for Zn availability from zineb and the repression of Cu toxicity was found in the solution culture studies, particularly with rough Lemon seedlings. This depression of Cu availability was beneficial to the roots and prevented the type of injury shown in Fig. 1. Rough lemon seedlings were more susceptible to this Cu injury to roots and the correction by the zineb than were the sour orange seedlings.

When zineb was present in the nutrient solution of experiments, no adverse effects were noted by visual observation of the foliage or roots except in the 0.5 ppm. Cu solution where low rates of zineb were used. In solution cultures when the Cu varied from none applied up to 0.5 ppm. of Cu applied, there was a lowering of Cu in the leaves and roots where zineb was applied. The other metals were not affected, with

the exception that the Zn content of both the leaves and roots increased with increasing rates of zineb. This increase in Cu content was not as great as when equivalent Zn from $ZnSO_4$ was used. This lowering of the Cu content of the plant was similar to that reported by Guinn and Joham (29) with di-Na ethylenediaminetetraacetate and tri-Na hydroxyethylethylene diaminetriacetate using cotton plants. These chelates, however, increased the Fe, but decreased the Cu, Mn, and Zn uptake in leaves and decreased the Cu, Zn and Fe in the roots.

An injury was observed on the rough lemon seedlings where zineb was not used, and to a lesser extent in the treatments where high Cu and low zineb were used. This injury could best be described as a cauliflower like knot at the tip of the seedling roots. The Cu content of the leaves and roots of seedlings having these knots was high compared to the plants where knots were not observed, which were the treatments receiving zineb. Applications of $ZnSO_4$ did not correct the Cu levels in the roots or the knot development. The Cu injury as reported by Smith and Specht (67) was the presence of dead roots.

Differences were not significant in the composition of the citrus leaves or roots of the seedlings grown in soil at different levels of Cu where zineb was used at the rate of 32 pounds per acre of the commercial material.

By chemical, x-ray diffraction, and biological analyses, no differences were observed between zineb not exposed and zineb exposed to direct sunlight up to 112 hours. In the biological analysis, the Cu content of the leaves of the seedlings, where exposed and not exposed zineb were used, was lowered to the same level. The Cu was not lowered

where zineb was not applied and was not lowered where different rates of Zn as $ZnSO_4$ were applied. This indicated that the lowering of the Cu content of the plant was due to the zineb and not to the excess absorption of Cu in the absence of applied Zn.

In the experiment where zineb and Mg or zineb, $ZnSO_4$ and Mg were used, differences were not noted due to treatments. These results were probably masked by previous fertility practices employed with this soil.

When zineb was incubated with the soil approximately 60 per cent of the Zn was calculated as being released. At the same time, significant amounts of NH_4-N could also be determined. This might have resulted from inhibition of nitrification by zineb as found by Jacques (38). The amount of extractable SO_4-S was not sufficient to conclude that it was being released from the zineb molecule. From the results obtained, it would appear that the Zn in zineb could be released in the soil but a conclusive statement could not be made about the degree of degradation of the zineb molecule because the absence of sufficient N and S in the extraction could be due to the need of these elements by the microbial population. The increase in NH_4-N where zineb was used indicated that either soil organic matter or zineb underwent considerable degree of degradation and that this NH_4-N was not oxidized to the NO_3-N form completely.

The microbial activity as indicated by the rate of evolution of CO_2-C, was lowered by the addition of zineb to soil containing sucrose. When inorganic salts equivalent to the Zn, N, and S in the zineb were applied along with zineb to the soil, this rate was only retarded during

the first four days, but regained and maintained the same rate as the soil containing sucrose during the remainder of the experiment, as shown in Fig. 4. The rates of evolution of CO_2-C were not significantly different after the first 10 days of incubation for the soil treatment containing sucrose with the inorganic supplement by itself, or with Cu added with or without zineb addition. Apparently the zineb reduced the microbial activity during the initial period but the presence of Cu prevented this reduction. These results, shown in Fig. 4, suggested that the zineb was inactivated during the first 10 days. These experiments were not of sufficient scope to show what actually occurred in the inactivation of the zineb.

The biological data suggested that sunlight did not change the zineb molecule. The microbiological experiments also indicated probable stability of the zineb. The x-ray diffraction data confirmed the stability of the zineb exposed to sunlight. Whereas nabam has been found to be rather unstable, zineb was rather stable in these studies.

CHAPTER VIII

SUMMARY

Two solution culture experiments were conducted with rough lemon seedlings using 0, 6.6, 46.6, 86.6 and 126.6 ppm. of zineb in the solution. In the first experiment, 0.5 ppm. of Cu was applied and Cu was omitted in the second experiment. From these experiments it was determined that the Cu content of the leaves and roots was lowered where zineb was applied. A root injury that looked under the microscope like a cauliflower head was observed on the tips of roots where zineb was not applied and to a lesser extent where the lower rates of zineb were applied. The injured seedlings were higher in Cu content of the leaves and roots than those which appeared not to be injured. The Zn content was increased in both leaves and roots as the concentration of zineb became greater. Content of Ca, Mg, K, P, Mn, and Fe in leaves and roots was not affected by the zineb.

Using sour orange seedlings, a solution culture experiment containing 0, 1, 7, 13, 19 ppm. of Zn as zineb, zineb that had been exposed to sunlight for 112 hours, and an equivalent treatment with $ZnSO_4$ was conducted. Due to a faulty aeration system, the data obtained from this experiment was somewhat limited. The Zn content of the leaves and roots was increased as the rates of the three materials were increased, but the uptake of Zn from $ZnSO_4$ was far greater than that from zineb or the exposed zineb. The Cu levels in the plant tissue were lowered

by the zineb. Other elements were not apparently affected by the zineb treatments. Roots developed better where zineb was present.

Chemical analysis by the method of Clarke *et al.* (9) for zineb gave approximately the same results, expressed as CS_2, for zineb that had been exposed for 0, 8, 32, 56, and 112 hours to direct sunlight. The X-ray diffraction analysis indicated that the crystalline structure of the commercial zineb was not altered after exposure for periods as stated above. The peak intensities for zineb, in order of magnitude, were spacings of 6.87, 4.82, 3.45, 3.03 and 4.30 Å. The carrier in the zineb was amorphous. The zineb was not removed from the carrier by chloroform extracts.

A biological assay of this exposed zineb was conducted at the rates of 7 and 13 ppm. of Zn with rough lemon seedlings. A treatment containing these same rates of Zn as $ZnSO_4$ was also included. Differences could not be noted for zineb exposed to sunlight from 8 to 112 hours but the treatments containing Zn from $ZnSO_4$ were slightly chlorotic and contained knots at their root tips, as did the checks. The Zn content of the leaves and roots of the treatments receiving exposed zineb was increased over that of the check while the Cu content was lowered below the check. The treatments receiving Zn from $ZnSO_4$ were very high in Zn and contained approximately the same Cu content as the checks. As before, other elements remained constant in the leaves and roots with zineb present in the nutrient solution.

A preliminary microbiological experiment was conducted using 50 ppm. of zineb and inorganic sources equivalent to the Zn, N, and S in zineb. These treatments were incubated with a Pomello soil, the same

soil inoculated with a zineb soil extract, or with an Arredondo soil extract. Soil analysis indicated the inoculated soils behaved similarly to that not inoculated. From the data obtained from this experiment, it was calculated that approximately 70 per cent of the Zn was released from the zineb into the soil. In another experimebt, similarly designed but using 100 ppm. of zineb and a higher level of sucrose as the energy source, it was calculated that approximately 60 per cent of the Zn was released from zineb. A small quantity of NH_4-N from zineb and organic matter decomposition was extracted, and indicated possible zineb degradation. From the CO_2-C evolved, it was noted that zineb did reduce the microbial activity in the soil where sucrose was the energy source. When inorganic N, S and Zn were added, the CO_2-C evolution was lowered only the first 4 days by zineb and then the 10-day interval rate was regained and maintained for the remainder of the experiment. The addition of Cu depressed the CO_2-C evolution initially. The effect of Cu on the zineb when it was added with the inorganic sources resulted in an increase of CO_2-C production for the first 4 days over the same treatment without Cu. This indicates that zineb was probably inactivated in the soil by Cu. Maban inhibited CO_2 evolution more than zineb applied at a higher rate of the dithiocarbamate.

Two experimebts were conducted to determine if an interaction occurred between Mg and the Zn from zineb and $ZnSO_4$ using sour orange seedlings. The rates of Mg and zineb applied were 65 and 32 pounds per acre respectively. Evidently Leon and Arredondo fine sands contained a relatively high level of Mg and a Mg.Zn interaction was not observed.

To determine the effect of Cu on zineb in the soil, an experiment was designed using sour orange seedlings and treatments of 200 pounds per acre of Cu, the same amount of Cu plus 32 pounds per acre of zineb, and the same amount of Cu plus nabam equivalent to the zineb. The differences between treatments were not significant. Analysis of the leachates from the treatments before the seedlings were planted indicated that zineb did not leach nor did it affect the amount of Cu entering the leachate. Dithiocarbamates were not found in the leachate.

In the second Mg experiment, where zineb and $ZnSO_4$ at the rate of 15 ppm of Zn were applied to the surface, soil samples were taken every inch to a depth of 4 inches. From the data obtained, it was noted that the downward movement of Zn from these sources was negligible over the 30 weeks that the experiment was conducted.

BIBLIOGRAPHY

1. Barratt, R. W., and Horsfall, J. G. 1947 Fungicidal action of metallic alkyl bisdithiocarbamates. Conn. Agr. Expt. Bul. 508.

2. Barratt, R. W. Raymond, W., and Horsfall, J. G. 1947 Fungicidal mechanism of disodium ethylene bisdithiocarbamate. Phytopath. 37:2.

3. Barrows, H. L. and Gammon, N., Jr. 1960 Effect of soil type and zinc concentration on growth, nutrient uptake, and magnesium translocation of seedling tung trees. Amer. Soc. Hort. Sci. Proc. 76:287-299.

4. Bartlett, F. D. and Neller, J. R. 1960 Turbidimetric determination of sulfate sulfur in soil extracts. Soil Sci. 90:201-204.

5. Benson, N. R., Batjer, L. P., and Chmelir, I. C. 1957 Response of some deciduous fruit trees to zinc chelates. Soil Sci. 84:63-75.

6. Bird, L. S., Ranney, C. D., and Watkins, G. M. 1957 Evaluation of fungicides mixed with the covering soil at planting as a control measure for cotton seedling-disease complex. Plant Disease Reptr. 41:165-173.

7. Boawn, L. C., Viets, F., Jr., and Crawford, C. L. 1956 Plant utilization of zinc from various types of zinc compounds and fertilizer materials. Soil Sci. 83:219-227.

8. Cheng, K. L. and Bray, R. H. 1953 Two specific methods of determining copper in soil and plant material. Anal. Chem. 25:655-659.

9. Clarke, D. G., Baum, H., Stanley, E. L. and Hester, W. F. 1951 Determination of dithiocarbamates. Anal. Chem. 23:1842-1846.

10. Conover, R. A. 1956 Certain fungicides injure cantaloupes. Sunshine State Agr. Res. Rpt. 1, (4):9-10.

11. Cox, R. S. 1956 Control of Helminthosporium blight diseases on sweet corn in south Florida. Phytopath. 46:112-115.

12. Cox, R. S., and Hayslip, N. C. 1956 Progress in the control of grey mold of tomato in south Florida. Plant Disease Reptr. 40:718-726.

13. Dean, H. A., and Sleeth, B. 1959 Control of fruit russeting in citrus. Rio Grande Val. Hort. Soc. 13:63-69.

14. Dekhuijzen, H M 1961 Transformation in plants of sodium dimethyldithiocarbamate into other fungitoxic compounds Nature 191 198-199

15 DeKock, P C , and Mitchell, R L 1957 Uptake of chelated metals by plants Soil Sci 84 55-62

16 Dimond, A E , Heuberger, J W , and Horsfall, J G 1943 A water soluble protectant fungicide with tenacity Phytopath 33 1095-1097

17 Enge, K G and Linn, M B 1952 Effect of spraying with zineb on the growth and zinc content of the tomato plant Phytopath 42 133-136

18. Fisher, F. E 1957 A summer application of zineb for the control of citrus fruit russet Citrus Indus 38(5) 5-6

19 Fisher, F E 1957 Zineb can cut cost of russet control Sunshine State Agr Res Rpt 2(2) 6

20 Fisher, F E 1957 Control of citrus fruit russet in Florida with zineb Phytopath 47(7) 433-437

21 Fisher, F E 1958 Use of zineb in Florida citrus groves for controlling russet and greasy spot Rio Grande Val Hort Soc J 12 3-6

22 Fiskell, J G A , Gammon, N , Jr., Yuan, T L and Zmeskal, O 1958 Colloidal properties of some Florida soils Soil Sci, Soc Amer Proc 22 339-343

23 Folin, O and Bell, R D 1917 Applications of a new reagent for the separation of ammonia I The colorimetric determination of ammonia in urine J Biol Chem 29 329-335

24 Genung, W G 1949 Dithane dust indicates effective control of melon worm and pickle worm Proc Fla State Hort Soc 62 130-132

25 Goksoyr, J 1955 Effect of some dithiocarbamoyl compounds on the metabolism of fungi Physiol Plantarum 8 719-835 (Chem Abs 50 7949-7950)

26 Griffiths, J T 1959 Results from the use of zineb in citrus groves during the 1957 and 1958 growing seasons Fla Entomol 42(2) 85-91

27 Griffiths, J T 1960 Field experience on some new miticides during the past twelve months on citrus in Florida Fla Entomol 43(1) 29-35

28 Griffiths, J T 1962 Field control with zineb and some other compounds for the control of rust mites on citrus in Florida Fla Entomol 45 71-76

29 Guinn, G and Joham, H E 1962 Effects of two chelating agents on adsorption and translocation of Fe, Cu, Mn, and Zn by the cotton plant Soil Sci 94 220-223

30 Halleck, F E, and Cochrane 1950 Effect of fungistatic agents on the bacterial flora of the rhizosphere Phytopath 40 715-718

31 Haupt, A W 1923 Cell structure and cell division in the cyanophyceae Bot Gaz 75 170-190

32 Heslop, R B, and Robinson, P L 1960 Inorganic Chemistry Elsevier Publishing Company New York pp 250, 496-497

33 Hester, W F (to Rohm to Haas Co.) 1943 Fungicidal compositions suitable for use on plants or seeds U S Pat 2,317,765 (Chem Abs 37 6082)

34 Heuberger, J W and Manns, T F 1943 Effect of zinc sulfate-lime on protective value of organic and copper fungicides against early blight of potato Phytopath 33 1113

35 Hildebrand, A A, McKeen, W E, and Roch, L W 1949 Row treatment of soil with tetramethythiuram and disulfide for control of blackroot of sugar-beet seedlings I Greenhouse tests Can J Research 27C 23-43

36 Hoagland, D R and Arnon, D I 1938 The water-culture method for growing plants without soil Calif Agr Expt Sta Circ 347.

37 Jackson, M L 1958 Soil Chemical Analysis Prentice-Hall, Inc., Englewood Cliffs, N J pp 65-66, 183-201, 219-221, 287-288, 396-406

38 Jacques, R P, Robinson, J B, and Chase, F E 1959 Effects of thiourea, ethyl urethan, and some dithiocarbamate fungicides on nitrification in Fox sandy loam soil Can J Soil Sci 39 235-243

39 Johnson, R B King, J R, and McBride, J J 1957 Zinab controls citrus rust mite (Phyllocoptruta oleivora) Fla State Hort Soc Proc, 70 38-48

40 Johnson, R B, Brogdon, J E, and King, J R 1958 Zineb for the control of rust mites--some timely observations and answers Citrus Indus 39(1) 326

41 Johnson, R B, King, J R, and McBride, J J 1958 Zineb controls citrus rust mite (Phyllocoptruta oleivora) Citrus Indus 39(2) 20-29

42 Kaslander, J, Sijpesteijn, A K, and Van der Kerk, G J M 1961 On the transformation of dimethyldithiocarbamate into its B-glucoside by plant tissues Biochem Biophys Acta 52 396-397

43. Kaslander, J., Sijpesteijn, A. K., and Van der Kerk 1962. On the transformation of the fungicide sodium dimethyldithiocarbamate into its alanine derivative by plant tissue. Biochem. Biophys. Acta. 60:417-419.

44. Klopping, H. L and Van der Kerk. 1951 Organic fungicides. V. Chemical constitution and fungistatic activity of aliphatic bisdithiocarbamates and isothiocyanates. Rec. trav. chem. 70:949-961.

45. Large, J. R, 1952 The pecan spray schedule for Florida. Proc. Ann. Conv. Southeast. Pecan Growers Assoc. 45.95-105. (Chem. Abs. 48 9604).

46. Levitsky, M. and Lowen, W. K. 1954 The analysis of manganese ethylenebis-(dithiocarbamate) compositions. J. Assoc. Offic. Agr. Chem. 37:555-557.

47. Ludwig, R. A. and Thorn, G. D. 1960 Chemistry and mode of action of dithiocarbamate fungicides. Advances in Pest Control Research Vol. 3. Edited by R. L. Metcalf. Interscience Publishers Inc. New York. pp. 219-252.

48. Magie, R. O. 1947 Experiments on gladiolus. Gladiolus Mag. 11(4) 14-20.

49. Magie, R. O. 1948 Curvularia and other important leaf and flower diseases of gladiolus in Florida. Gladiolus Mag. 12(1):13-16.

50. Magie, R. O. 1951 Botrytis and curvularia diseases of gladiolus. North Am. Gladiolus Council Bul. 27:85-90.

51. Manten, A., Klopping, H. L. and Van der Kerk, J. M. 1950 Organic fungicides. I. Antimicrobial spectrum of the antifungal substance tetraethylthiuram disulfide. Antonie van Leeuwenhoek. J. Microb. Serol. 16:45-55. (Chem. Abs. 44.7001).

52. Martell, A. E. 1957 The chemistry of metal chelates in plant nutrition Soil Sci. 84:13-26.

53. Mehlich, A. 1956 Improvements in the colorimetric magnesium and ammonium methods with sodium polyacrylate. Assoc. Official Agr. Chem. 39:518-523.

54. Merrill, S., Jr. Potter, G. F., and Brown, R. T. 1953 Responses of tung trees on Lakeland fine sand to less common elements. Proc. Am. Soc. Hort. Sci. 62:94-102.

55. Miller, P. R. 1957 Zineb--improved summer control of citrus fruit russet. Citrus Indus. 38(11):7-9, 27-28.

56. Moore, W. D., and Conover, R. A. 1955 Chemical soil treatments for control of Rhizoctonia of snap beans. Plant Disease Reptr. 39:103-105.

57. Palmiter, D. H. 1949 The effects of fermate on the yield of McIntosh apples. Phytopath. 39:18.

58. Ranney, C. D., and Bird, L. S. 1956 Greenhouse evaluation of in-the-furrow fungicides at two temperatures as a control measure for cotton seedling necrosis. Plant Disease Reptr. 40:1032-1040.

59. Reid, W. J., Jr. 1951 Zinc ethylenebisdithiocarbamate toxic to pickleworm and melonworm J. Econ. Entomol. 44:817-818.

60. Reid, W. J., Jr., and Cuthbert, F. P., Jr. 1955 Test of insecticides for control of the pickleworm and associated insects of cucumbers and squash, 1948-1951. U. S. Bur. Entomol. and Plant Quarantine E-856:1-34.

61. Rohwer, S. A. 1950 Common names for five commercially available fungicidal chemicals. Phytopath. 40:118.

62. Rosenthal, I., Carlson, G. L. and Stanley, B. L. 1953 Interferences in the analysis of zinc ethylenebis(dithiocarbamate). J. Assoc. Offic. Agr. Chem. 36:1170-1182.

63. Ruehle, G. D. 1947 Recent spray tests for control of potato late blight in sub-tropical Florida. Amer. Potato J. 24:299-307.

64. Sandell, E. B. 1950 Colorimetric Determination of Traces of Metals ed. 2. Interscience Publishers, Inc., New York. pp. 375-380, 430-433, 616-626.

65. Sato, T. and Tandzawa, C. 1961 Decomposition of zineb applied to plants. Nogyo Gijutsu Kenkyusho Hokoku Ser. C, 12:181-187. (Chem. Abs. 55:15812).

66. Seatz, L. F. 1960 Zinc availability and uptake by plants as affected by the calcium and magnesium saturation and phosphorus content of the soil. Trans. 7th Int. Congr. Soil Sci. 2:271-280

67. Smith, P. F. and Specht, A. W. 1952 Heavy-metal nutrition in relation to iron chlorosis of citrus seedlings. Fla. Sta. Hort. Soc. Proc. 65:101-108.

68. Stewart, I., and Leonard, C. D. 1952 Chelates as sources of iron for plants growing in the field. Science 116:564-566.

69. Thompson, W. L., Simanton, W. A., and Johnson, R. B. 1960 Citrus insect control for the winter 1960-1961. Citrus Indus. 41(11):3, 18.

70. Tiffin, L. O., Brown, J. C., and Krauss, R. W. 1960 Differential absorption of metal chelate components by plant roots. Plant Physiol. 35:362-367.

71. Tisdale, W. H. and Williams, I. (to E. I. duPont de Nemours & Co.) 1934 Disinfectant and fungicide. U. S. Pat. 1,972,961.

72. Viles, F. J. 1940 Field determinations of carbon disulfide in air J. Ind. Hyg. Toxicol. 22:188-196. (Chem. Abs. 34:4356).

73. Wilson, J. D. and Sleesman, J. P. 1947 Some of the newer pesticides damage plants. Ohio Agr. Expt. Sta. Farm and Home Res. 32:58-63. (Chem. Abs. 41:6362).

74. Wilson, J. D., and Sleesman, J. P. 1948 Pesticides--a study of their effects on the growth and transpiration of cucumber, tomato, and potato plants. Ohio Agr. Expt. Sta. Bul. 676.

APPENDIX

APPENDIX

TABLE 1

Comparison of the modified flame and the EDTA titration methods for Ca

Replicates	Flame Photometer[a]			EDTA Titration[b]		
	Leaf sample					
	1	2	3	1	2	3
	Ca content	%				
1	1.80	1.68	0.70	1.86	1.78	0.79
2	1.74	1.58	0.77	1.86	1.86	0.77
3	1.80	1.65	0.72	1.92	1.72	0.76
Ave.	1.78	1.64	0.73	1.88	1.79	0.77

[a]Modified compensating solution described in the text.
[b]Method of Jackson (37).

APPENDIX

TABLE 2

Comparison of the modified flame and Mehlich's thiazole yellow-polyacrylate methods for Mg

Leaf Sample	Flame Photometer*	Thiazole yellow Colorimetric*
	Mg content, %	
1	0.35	0.32
2	.35	.31
3	.44	.40
4	.43	.44
5	.44	.42
6	.43	.29
7	.30	.26
8	.30	.27
9	.40	.45
10	.52	.46
11	.42	.41
Ave.	0.40	0.37

*See text.

APPENDIX

TABLE 3

Sequence of solutions used in staining tissue

Step	Solution	Contact in Minutes
1	xylene	15
2	xylene.ethanol	5
3	ethanol	3
4	95% ethanol	2
5	70% ethanol	2
6	50% ethanol	2
7	Water, running	
8	1% tannic acid	10
9	Water, running	
10	3% ferric chloride	3
11	Water, running	
12	1% safranin	10 at 52°C
13	Water, running	
14	70% ethanol	1
15	95% ethanol	1
16	fast green	
17	95% ethanol	1
18	100% ethanol	3-5
19	xylene	2
20	xylene	2
21	xylene	5

APPENDIX

TABLE 4

Plant measurements and chemical composition of rough lemon seedlings grown in solution culture containing several levels of zineb in the presence of 0.5 ppm. of Cu

Replicates	Level of Zineb in Solution Culture ppm. 0	6.6	46.6	86.6	126.6
	Fresh weight of new foliage, g.				
1	7.0	15.3	4.7	25.1	15.3
2	17.2	9.9	58.4	12.7	21.9
3	4.9	12.9	14.4	12.4	30.5
Ave.	9.7	12.7	25.8	16.7	22.6
	Increased weight of entire seedling, g.				
1	8.8	23.6	6.5	44.1	26.9
2	25.1	14.8	82.6	17.3	34.5
3	10.3	20.0	22.3	17.8	45.0
Ave.	14.7	1.95	37.1	26.1	38.8
	Number of new leaves				
1	32	71	40	85	111
2	96	68	172	56	93
3	28	54	81	50	92
Ave.	52	64	98	64	99
	Green weight of new leaves, g.				
1	6.0	10.9	4.8	17.5	11.6
2	13.1	6.9	40.0	10.0	17.2
3	3.8	9.8	11.3	10.0	23.0
Ave.	7.6	9.2	18.4	1.26	13.9

TABLE 4--Continued

Replicates	Level of Zineb in Solution Culture ppm.				
	0	6.6	46.6	86.6	126.6
			Dry weight of new leaves, g		
1	1.5	3.5	1.3	5.1	3.1
2	4.5	2.1	12.7	2.9	4.9
3	1.4	3.3	3.6	2.9	6.9
Ave.	2.5	3.0	5.9	3.6	5.0
			Increase in root volume, cc.		
1	1.8	5.6	1.4	8.4	8.1
2	4.8	3.7	19.5	3.3	10.0
3	3.5	4.8	6.8	3.2	10.8
Ave.	3.4	4.7	9.2	5.0	9.6
			Dry weight of roots, g.		
1	1.2	2.7	1.4	3.8	3.2
2	2.3	2.3	6.0	1.9	2.9
3	1.6	2.7	2.4	1.7	3.4
Ave.	1.7	2.6	3.3	2.5	3.2
			Ca content of new leaves, %		
1	0.97	0.68	0.62	1.07	0.92
2	0.76	0.74	1.07	0.76	0.78
3	0.99	0.77	0.62	0.92	1.06
Ave.	0.91	0.73	0.77	0.92	0.94
			P content of new leaves, %		
1	0.26	0.28	0.27	0.27	0.26
2	0.22	0.16	0.27	0.24	0.28
3	0.25	0.18	0.17	0.26	0.22
Ave.	0.24	0.21	0.24	0.26	0.25

TABLE 4--Continued

Replicates	Level of Zineb in Solution Culture ppm.				
	0	6.6	46.6	86.6	126.6
			Mg content of new leaves, %		
1	0.34	0.28	0.24	0.19	0.31
2	0.19	0.20	0.17	0.22	0.21
3	0.20	0.18	0.19	0.20	0.21
Ave.	0.24	0.22	0.20	0.20	0.24
			K content of new leaves, %		
1	1.96	1.79	2.18	2.08	2.21
2	1.54	1.57	.80	1.78	2.31
3	1.90	1.64	1.51	2.04	1.79
Ave.	1.80	1.67	1.50	1.97	2.10
			Zn content of new leaves, ppm.		
1	20.5	14.7	25.9	61.4	83.3
2	13.3	27.5	26.4	73.0	88.4
3	26.6	25.2	21.8	58.9	94.1
Ave.	20.0	21.8	24.7	64.4	88.6
			Zn uptake by new leaves.		
1	30.8	51.5	33.7	313.4	258.3
2	59.9	57.8	335.8	212.0	432.6
3	37.0	76.5	78.5	171.1	649.9
Ave.					
			Cu content of new leaves, ppm.		
1	4.3	4.7	4.5	2.3	3.4
2	6.8	5.2	2.7	2.0	2.3
3	11.7	4.7	2.7	2.6	1.9
Ave.	7.6	4.9	3.3	2.3	2.5

TABLE 4--Continued

Replicates	Level of Zinc in Solution Culture ppm.				
	0	6.6	46.6	86.6	126.6
			Cu uptake by new leaves,		
1	6.5	16.8	5.6	11.7	10.6
2	30.6	10.9	34.3	5.8	11.3
3	16.4	15.5	9.7	7.6	13.1
Ave.	17.8	14.4	16.6	8.4	11.7
			Mn content of new leaves, ppm.		
1	35	15	6	13	20
2	12	11	10	14	12
3	18	9	12	14	8
Ave.	22	17	9	14	13
			Fe content of new leaves, ppm.		
1	97	55	58	64	54
2	86	54	67	58	65
3	66	50	65	54	76
Ave.	83	53	63	59	72
			Ca content of roots, %		
1	0.66	0.39	0.64	0.75	0.70
2	0.47	0.58	0.50	0.53	0.64-
3	0.54	0.39	0.50	0.69	0.78
Ave.	0.56	0.39	0.55	0.64	0.70
			P content of roots, %		
1	0.24	0.19	0.42	0.14	0.16
2	0.19	0.25	0.07	0.30	0.16
3	0.30	0.30	0.26	0.28	0.13
Ave.	0.24	0.25	0.25	0.24	0.15

TABLE 4--Continued

Replicates	Level of Zineb in Solution Culture ppm.				
	0	6.6	46.6	86.6	126.6
			Mg content of roots, %		
1	0.16	0.24	0.15	0.34	0.35
2	0.26	0.18	0.19	0.14	0.43
3	0.15	0.19	0.30	0.18	0.26
Ave.	0.19	0.20	0.21	0.22	0.35
			K content of roots, %		
1	0.67	0.68	0.72	1.60	1.22
2	0.63	0.47	0.80	0.68	1.34
3	0.67	0.51	0.67	0.81	1.26
Ave.	0.66	0.55	0.73	1.03	1.28
			Zn content of roots, ppm.		
1	62	65	217	736	720
2	15	47	491	716	909
3	30	50	323	842	1,010
Ave.	36	54	344	765	880
			Zn adsorption by roots,		
1	75	176	304	2,798	2,320
2	35	108	2,946	1,364	2,641
3	48	135	775	1,434	3,434
Ave.	53	140	1,342	1,865	2,798
			Cu content of roots, ppm.		
1	160	83	42	33	27
2	193	69	32	23	25
3	264	80	43	35	15
Ave.	205	77	39	30	22

TABLE 4--Continued

Replicates	Level of Zinc in Solution Culture ppm.				
	0	6.6	46.8	86.6	128.6
	Cu adsorption by roots,				
1	192	224	59	125	88
2	444	159	192	44	73
3	423	216	103	60	51
Ave.	353	200	118	78	70
	Mn content of roots, ppm.				
1	59	29	15	25	25
2	41	21	27	37	26
3	51	26	25	41	56
Ave.	50	25	22	34	36
	Mn adsorption by roots,				
1	71	78	21	95	80
2	94	48	162	70	75
3	82	70	60	70	190
Ave.	82	65	81	78	115
	Fe content of roots, ppm.				
1	604	839	718	1,010	1,630
2	458	489	932	1,650	1,530
3	201	724	1,490	1,210	1,580
Ave.	420	684	1,047	1,290	1,513
	Fe adsorption by roots,				
1	725	2,263	1,052	3,810	4,560
2	1,023	1,107	5,602	3,130	4,470
3	678	1,958	3,590	2,050	5,420
Ave.	809	1,778	3,415	2,997	4,817

APPENDIX

TABLE 5

Plant measurements and chemical composition of rough lemon seedlings grown in solution culture containing several levels of zineb without Cu added

Replicates	Level of Zineb in Solution Culture ppm.				
	0	6.6	46.6	86.6	126.6
	Fresh weight of new foliage, g.				
1	5.1	6.6	30.5	4.2	12.9
2	8.9	14.5	13.3	10.6	14.5
3	3.3	7.1	14.7	23.0	20.9
Avg.	5.7	9.4	19.5	12.6	16.1
	Increased weight of entire seedling, g.				
1	7.0	9.2	49.6	5.7	19.3
2	15.4	19.6	17.7	15.5	20.3
3	5.9	9.4	20.2	38.1	27.9
Avg	9.4	12.5	29.2	34.8	22.5
1	**Number of new leaves**				
1	39	80	112	20	70
2	80	84	76	79	84
3	28	46	63	92	71
Avg	47	70	84	84	75
	Dry weight of new leaves, g.				
1	1.22	1.93	3.87	1.04	2.99
2	2.43	3.64	3.53	2.49	3.14
3	0.79	1.77	4.09	5.46	4.05
Avg.	1.48	2.41	4.49	3.00	3.39

TABLE 5--Continued

Replicates	Level of Zineb in Solution Culture ppm.				
	0	6.6	46.6	86.6	126.6
	Increase in root volume, cc.				
1	1.8	3.0	16.3	1.3	4.8
2	4.6	2.9	4.1	4.8	4.8
3	0.7	1.8	4.4	12.0	7.2
Avg.	2.4	2.6	8.3	6.0	5.6
	Dry weight of roots, g.				
1	1.42	1.94	3.97	0.99	2.15
2	1.90	1.96	2.57	1.96	1.76
3	1.12	1.54	2.30	4.37	2.66
Avg.	1.48	1.81	2.95	2.44	2.19
	Ca content of new leaves, %				
1	1.23	0.77	1.28	0.76	0.78
2	0.54	0.87	0.81	1.02	1.18
3	0.87	0.62	0.59	1.10	0.74
Avg.	0.88	0.75	0.89	0.96	0.90
	P content of new leaves, %				
1	0.22	0.32	0.24	0.23	0.30
2	0.22	0.27	0.25	0.35	0.36
3	0.27	0.22	0.24	0.27	0.36
Avg.	0.24	0.27	0.24	0.25	0.34
	Mg content of new leaves, %				
1	0.23	0.23	0.22	0.20	0.21
2	0.12	0.25	0.22	0.27	0.34
3	0.39	0.22	0.16	0.31	0.25
Avg.	0.25	0.23	0.20	0.26	0.27

TABLE 5--Continued

Replicates	Level of Zineb in Solution Culture ppm.				
	0	6.6	46.6	86.6	126.6
	K content of new leaves, %				
1	1.91	2.29	1.90	1.85	1.75
2	1.83	2.09	1.56	2.19	1.75
3	2.09	1.79	1.69	1.85	2.09
Avg	1.94	2.06	1.72	1.96	1.86
	Zn content of new leaves, ppm				
1	40	18	60	59	60
2	20	35	47	52	113
3	15	17	44	113	92
Avg	25	23	50	75	88
	Zn uptake by new leaves, μg.				
1	48	33	353	61	179
2	49	127	166	129	385
3	12	30	180	617	373
	Cu content of new leaves, ppm				
1	8.4	3.2	3.5	2.4	3.3
2	23.8	5.6	2.4	2.1	2.2
3	4.1	5.4	2.6	1.7	1.5
Avg.	13.1	4.7	2.8	2.1	2.3
	Cu uptake by new leaves, μg.				
1	10.2	5.9	20.5	2.5	9.9
2	57.9	20.4	8.5	5.2	6.9
3	3.2	9.6	12.5	9.3	6.1
Avg.	23.8	12.0	13.8	5.7	7.6

TABLE 5.--Continued

Replicates	Level of Zineb in Solution Culture, ppm.				
	0	6.6	46.6	86.6	126.6
	Mn content of new leaves, ppm.				
1	13	16	32	22	14
2	24	9	15	11	14
3	10	11	8	13	15
Avg.	16	12	18	15	14
	Fe content of new leaves, ppm				
1	93	101	72	57	60
2	41	43	46	50	39
3	63	43	58	65	86
Avg	66	62	59	57	62
	Ca content of roots, %				
1	0.77	0.86	0.65	0.78	0.73
2	0.58	0.63	0.63	0.75	0.56
3	0.86	0.57	0.41	0.67	0.44
Avg	0.77	0.62	0.57	0.74	0.58
	P content of roots, %				
1	0.34	0.32	0.11	0.20	0.25
2	0.33	0.36	0.11	0.41	0.15
3	0.43	0.34	0.20	0.11	0.15
Avg.	0.37	0.34	0.14	0.23	0.18
	Mg content of roots, %				
1	0.12	0.12	0.20	0.10	0.17
2	0.13	0.17	0.25	0.17	0.12
3	0.25	0.10	0.26	0.24	0.42
Avg	0.17	0.13	0.23	0.17	0.24

TABLE 5--Continued

Replicates	Level of Zinc in Solution Culture ppm.				
	0	6.6	46.6	86.6	126.6
	K content of roots, %				
1	0.68	0.43	1.53	0.64	0.89
2	0.61	0.90	0.88	0.89	0.64
3	1.28	0.51	0.72	1.01	0.86
Avg.	0.94	0.61	1.04	0.85	0.80
	Zn content of roots, ppm.				
1	15	105	536	687	1,280
2	11	137	360	799	1,080
3	14	75	459	623	1,100
Avg.	13	106	452	703	1,153
	Zn adsorption by roots, μg.				
1	21	202	2,120	680	2,760
2	21	269	952	1,560	1,900
3	16	115	1,060	2,720	2,920
Avg.	19	195	1,368	1,653	2,527
	Cu content of roots, ppm.				
1	29	11	7.2	7.7	7.1
2	24	15	8.4	3.6	6.2
3	26	26	4.0	5.5	7.4
Avg.	27	17	6.5	5.6	6.9
	Cu adsorption by roots, μg.				
1	41	21	29	8	15
2	46	29	22	7	11
3	30	40	9	24	20
Avg.	39	30	20	13	15

TABLE 5--Continued

Replicates	Level of Zineb in Solution Culture ppm.				
	0	6.6	46.6	86.6	126.6
	Mn content of roots, ppm.				
1	36	34	37	24	48
2	44	38	28	42	29
3	74	23	27	36	31
Avg	51	32	31	34	36
	Fe content of roots, ppm.				
1	115	746	348	1,680	933
2	270	530	1,000	1,130	894
3	356	161	894	456	872
Avg.	247	479	747	1,089	900
	Fe adsorption by roots, μg.				
1	163	1,450	1,380	1,670	2,010
2	513	1,040	2,570	2,220	1,570
3	389	227	2,050	1,980	2,320
Avg.	358	906	2,000	1,957	1,967
	Mn adsorption by roots, μg.				
1	51	66	147	24	105
2	84	75	72	82	51
3	83	35	62	157	83
Avg.	73	59	94	88	80

APPENDIX

TABLE 6

Comparison of sunlight-exposed Zineb and $ZnSO_4$ at two levels of Zn showing plant measurements and chemical composition of rough lemon seedlings grown in solution culture

Repl.	Check	$ZnSO_4$		Zineb, Hours Exposed to Sunlight							
				8		32		56		112	
	Rate of Zn Applied, ppm.										
	0	0	7	13	7	13	7	13	7	13	7	13
	Fresh Weight of New Foliage, g.											
1	12.2	12.7	5.4	2.0	19.1	9.3	8.8	3.0	19.1	16.5		23.8
2	6.6	8.7	6.7	3.7	29.1	8.6	8.1	32.4	29.1	12.7	28.9	12.4
3	2.4	7.9	16.9	3.3	18.5	11.1	29.3	13.0	14.2	5.9	11.2	10.5
4	7.6	4.4	4.8	2.7	19.8	22.4	32.3	31.3	10.8	14.3	9.2	16.3
Ave.	7.2	8.4	8.5	2.9	21.6	12.8	17.1	19.9	18.3	12.9	12.3	15.8
	Increased Weight of Entire Seedling, g.											
1	17.1	18.0	10.0	3.6	31.9	11.8	11.2	5.2	28.2	23.7	27.3	38.7
2	9.3	11.4	14.3	6.7	44.6	12.4	10.3	61.0	45.2	17.2	52.8	16.1
3	2.4	12.1	26.0	5.1	29.7	17.6	41.0	18.2	23.4	8.4	16.4	18.2
4	10.3	5.2	6.9	1.9	29.0	38.9	38.4	47.4	15.9	20.6	12.8	27.2
Ave.	9.8	11.7	14.3	4.3	33.8	20.2	25.2	33.0	28.2	18.0	27.3	25.0
	Number of New Leaves											
1	74	75	50	39	66	22	61	52	101	90	76	75
2	63	73	55	32	106	52	71	101	70	54	97	94
3	63	57	70	26	91	57	88	71	46	62	66	64
4	77	50	42	28	89	100	89	95	70	75	66	59
Ave.	69	64	54	31	88	58	77	80	72	70	76	73

TABLE 6--Continued

Rep.	Check	ZnSO4		Zineb, Hours Exposed to Sunlight							
				8		32		56		112	
	Rate of Zn Applied, ppm.										
	0	0	7	13	7	13	7	13	7	13	7	13
	Dry Weight of New Leaves, g.											
1	3.1	3.0	1.3	0.5	5.0	1.8	1.6	0.7	4.3	3.3	3.9	5.4
2	1.7	2.0	2.0	1.0	6.7	2.1	2.1	7.5	7.2	2.6	6.7	3.2
3	0.6	1.8	4.0	0.9	4.9	2.9	5.9	3.3	2.9	1.5	3.0	2.6
4	1.7	0.9	1.2	0.7	4.7	4.6	5.6	7.5	2.6	4.3	2.2	3.6
Avg.	1.8	1.9	2.1	0.8	5.3	2.8	4.3	4.8	4.2	2.9	3.9	3.7
	Increase in Root Volume, cc.											
1	3.2	3.9	2.3	0.5	9.6	1.6	1.3	1.7	7.3	6.9	9.1	10.9
2	4.2	2.5	4.9	1.2	12.1	1.8	2.0	26.3	11.8	3.2	21.1	2.6
3	0.1	2.3	4.4	0.6	9.1	6.0	10.5	4.2	5.9	1.6	3.8	2.4
4	1.8	0.6	0.7	0.1	7.4	13.5	10.6	11.7	3.4	1.8	2.3	7.9
Avg.	2.1	2.3	3.1	0.6	3.6		6.1	11.0	7.1	3.4	9.1	6.0
	Dry Weight of Roots, g.											
1	2.4	2.5	1.9	1.8	3.4	2.8	1.2	1.4	2.8	3.2	1.5	4.9
2	1.8	1.9	1.9	1.3	3.8	1.8	2.3	5.9	4.8	2.3	4.7	1.9
3	1.0	1.3	2.6	1.5	3.0	3.5	4.2	2.4	3.2	1.7	2.0	3.0
4	1.1	1.3	1.2	0.6	2.8	3.6	3.7	4.4	1.8	2.6	1.6	3.0
Avg.	1.6	1.8	1.9	1.3	3.2	2.9	2.8	3.8	3.2	2.6	2.4	3.2

TABLE 6—Continued

Reps.	Check		ZnSO4		Zineb, Hours Exposed to Sunlight							
					8		32		56		112	
	Rate of Zn Applied, ppm											
	0	0	7	13	7	13	7	13	7	13	7	13
	Cu Content of Leaves, %											
1	0.77	0.77	1.23	1.22	1.00	0.91	1.25	0.84	0.77	0.85	0.69	1.08
2	0.58	0.79	0.68	0.75	1.29	0.81	0.98	1.34	1.07	0.87	1.15	0.97
3	1.42	1.05	0.63	0.93	0.95	0.71	0.92	0.69	0.98	1.32	0.74	0.80
4	0.66	0.89	0.77	1.14	0.80	1.12	0.99	1.44	0.84	0.63	0.86	0.98
Av.	0.86	0.86	0.83	1.01	0.89	0.89	1.04	1.08	0.93	0.94	0.69	0.96
	P Content of Leaves, %											
1	0.22	0.20	0.27	0.21	0.23	0.29	0.41	0.27	0.29	0.25	.23	0.24
2	.23	.23	.20	.15	.24	.24	.22	.25	.20	.25	.20	.27
3	.25	.24	.25	.19	.21	.22	.25	.29	.21	.20	.24	.26
4	.23	.33	.20	.19	.23	.28	.23	.22	.26	.28	.26	.28
Av.	.23	.25	.23	.18	.23	.26	.28	.26	.24	.24	.23	.26
	Mg Content of Leaves, %											
1	0.25	0.23	0.33	0.44	0.26	0.36	0.40	0.33	0.25	0.23	0.23	0.21
2	.12	.31	.14	.24	.25	.26	.28	.26	.21	.20	.20	.23
3	.34	.27	.26	.37	.25	.21	.28	.21	.29	.25	.21	.23
4	.21	.35	.25	.28	.15	.24	.19	.22	.23	.19	.27	.27
Av.	.23	.29	.24	.33	.23	.27	.29	.25	.24	.22	.23	.23

TABLE 6--Continued

Reps.	Check		$ZnSO_4$		Zineb, Hours Exposed to Sunlight							
					8		32		56		112	
	Rate of Zn Applied, ppm											
	0	0	7	13	7	13	7	13	7	13	7	13
	K Content of Leaves, %											
1	1.68	1.64	1.95	2.12	1.64	2.02	2.68	2.47	1.08	1.88	2.06	1.79
2	2.30	1.81	1.52	1.77	1.95	1.74	1.67	1.75	1.72	2.73	2.29	1.80
3	3.12	2.28	1.81	1.72	1.53	1.83	1.84	1.65	2.08	1.83	1.79	1.77
4	1.97	2.48	1.67	1.87	1.78	2.26	1.79	1.91	2.26	2.01	2.11	1.43
Ave.	2.27	2.05	1.74	1.87	1.72	1.96	2.00	1.94	1.78	2.11	2.06	1.70
	Zn Content of Leaves, ppm											
1	34	13	108	48	76	54	60	41	38	39	71	99
2	38	15	299	84	73	43	83	89	57	53	120	51
3	37	21	139	76	56	63	78	53	55	48	38	45
4	18	19	69	332	42	78	73	54	42	74	55	74
Ave.	32	17	154	135	62	60	73	59	48	53	71	67
	Cu Content of Leaves, ppm											
1	9.7	6.3	10.5	11.9	2.2	3.6	4.8	7.3	2.9	2.4	3.3	1.5
2	11.1	7.1	8.6	10.9	2.3	4.4	7.2	1.2	1.4	2.0	2.3	2.9
3	22.1	7.9	7.7	8.5	2.6	2.6	2.2	1.6	3.2	4.0	3.4	1.7
4	10.1	9.3	8.6	12.7	2.2	1.5	1.5	1.8	2.9	2.3	4.1	1.6
Ave.	13.2	7.6	8.8	11.0	2.2	3.0	3.9	3.0	2.6	2.7	3.3	1.9

TABLE 6—Continued

Reps.	Check		ZnSO4		Zineb, Hours Exposed to Sunlight							
					8		32		56		112	
	Rate of Zn Applied, ppm.											
	0	0	7	13	7	13	7	13	7	13	7	13
	Mn Content of Leaves, ppm											
1	18	5	23	24	22	22	34	10	9	11	15	17
2	11	18	21	23	13	23	6	14	13	9	13	9
3	13	7	23	23	19	16	15	6	20	28	12	21
4	10	23	21	30	6	18	11	7	16	13	19	17
Avg	13	13	22	25	12	20	16	9	14	15	15	16
	Fe Content of Leaves, ppm											
1	49	56	47	52	64	79	97	82	70	46	61	11
2	44	49	61	60	37	57	28	72	60	22	54	43
3	62	34	63	105	18	69	71	45	67	56	69	40
4	52	47	53	58	62	79	62	14	56	62	55	49
Avg	52	46	56	69	45	71	64	53	63	44	61	36
	Ca Content of Roots, %											
1	0.48	0.49	0.77	0.88	0.64	0.57	0.67	1.01	0.65	0.55	0.53	0.64
2	0.63	0.72	0.74	0.72	0.71	0.59	0.54	0.75	0.61	0.54	0.65	0.68
3	0.78	0.80	0.64	0.82	0.51	0.64	0.65	0.58	0.45	0.73	0.45	0.53
4	0.72	1.77	0.31	0.61	0.68	0.64	0.69	0.62	0.67	0.53	0.65	0.59
Avg	0.65	0.82	0.76	0.76	0.61	0.61	0.64	0.74	0.60	0.59	0.60	0.61

TABLE 6--Continued

Reps	Check		ZnSO$_4$		Zineb, Hours Exposed to Sunlight							
					8		32		56		112	
	Rate of Zn Applied, ppm.											
	0	0	7	13	7	13	7	13	7	13	7	13
	P Content of Roots, %											
1	0.25	0.12	0.41	0.46	0.22	0.17	0.19	0.36	0.28	0.25	0.21	0.20
2	0.32	0.29	0.34	0.19	0.24	0.31	0.12	0.45	0.20	0.14	0.21	0.43
3	0.20	0.26	0.52	0.34	0.23	0.25	0.23	0.10	0.21	0.35	0.33	0.19
4	0.29	0.30	0.67	0.75	0.23	0.27	0.24	0.26	0.38	0.16	0.30	0.25
Av.	0.28	0.24	0.48	0.36	0.23	0.25	0.20	0.29	0.27	0.23	0.26	0.27
	Mg Content of Roots, %											
1	0.18	0.21	0.16	0.12	0.21	0.10	0.18	0.34	0.18	0.15	0.18	0.21
2	0.11	0.10	0.21	0.10	0.26	0.09	0.27	0.41	0.20	0.29	0.33	0.16
3	0.17	0.10	0.32	0.12	0.12	0.24	0.21	0.23	0.12	0.18	0.15	0.11
4	0.14	0.10	0.13	0.20	0.15	0.39	0.23	0.34	0.17	0.32	0.17	0.20
Av.	0.15	0.13	0.20	0.27	0.18	0.20	0.22	0.33	0.18	0.24	0.21	0.17
	K Content of Roots, %											
1	0.50	0.47	0.63	0.60	1.03	0.54	0.77	0.74	1.04	0.90	0.85	0.97
2	0.48	0.52	0.82	0.58	1.26	0.67	0.64	1.56	0.82	0.74	1.50	0.82
3	0.54	0.60	0.68	0.60	0.54	0.83	0.74	0.80	0.54	0.78	0.60	0.55
4	0.66	0.63	0.55	0.58	0.83	1.28	1.04	1.31	0.66	0.78	0.82	0.97
Av.	0.55	0.56	0.67	0.59	0.92	0.88	0.80	1.10	0.75	0.80	0.85	0.83

TABLE 6--Continued

Reps	Check	ZnSO$_4$		Zineb, Hours Exposed to Sunlight							
				8		32		56		112	
	Rate of Zn Applied, ppm.										
	0	0	7	13	7	13	7	13	7	13	7	13
	Zn Content of Roots, ppm											
1	13	12	900	941	390	357	761	242	454	468	566	611
2	11	12	980	1,020	504	720	410	568	396	524	530	640
3	12	15	1,340	969	486	411	534	655	307	559	572	408
4	11	19	888	1,100	351	862	463	718	551	667	599	692
Avg	12	14	1,041	1,007	433	588	542	546	427	554	566	588
	Cu Content of Roots, ppm											
1	19	15	18	10	6	7	11	11	7	3	8	4
2	20	21	29	24	7	9	5	2	3	7	3	5
3	36	17	16	20	3	6	7	3	6	6	9	5
4	23	19	15	34	6	3	6	5	9	4	11	3
Avg	22	18	20	22	5	6	7	5	6	5	8	4
	Fe Content of Roots, ppm											
1	334	382	453	290	615	1,330	908	657	868	499	662	343
2	334	288	509	753	426	1,040	1,300	467	416	858	474	724
3	498	374	1,230	871	991	627	504	2,060	1,374	1,150	602	700
4	346	404	521	592	818	859	470	646	997	1,070	911	906
Avg	[illegible]	362	693	627	712	964	796	958	914	894	662	668

TABLE 6--Continued

Reps	Check		ZnSO$_4$		Zineb, Hours Exposed to Sunlight							
					8		32		56		112	
	Rate of Zn Applied, ppm											
	0	6	7	13	7	13	7	13	7	13	7	13
	Mn Content of Roots, ppm											
1	21	21	21	9	38	74	94	17	30	23	37	22
2	19	16	18	7	32	47	41	37	25	21	18	29
3	7	19	31	14	36	27	27	34	26	39	36	26
4	23	14	17	16	20	14	50	26	52	30	57	29
Avg.	17	18	22	12	32	40	53	28	33	28	37	26

APPENDIX

TABLE 7

Microbiological study of degradation of zineb by comparison with the equivalent inorganic salt addition and the control using Pomello fine sand, where A is not inoculated, B inoculated with extract from Leon fine sand and C inoculated with extract from Arredondo fine sand

Replicates	Zineb			Inorganic Zn, N, and S Equivalent to the Zineb			Control		
	A	B	C	A	B	C	A	B	C
				Zn extracted from soil, ppm.					
1	3.8	3.3	3.1	8.4	8.7	7.3	0.2	0.2	0.2
2	3.9	3.7	3.4	8.7	7.6	8.3	0.1	0.2	0.1
3	3.1	3.5	4.9	8.1	8.6	7.6	0.2	0.2	0.3
4	3.6	3.1	3.3	9.0	9.4	9.6	0.2	0.2	0.1
Ave.	3.4	3.0	3.2	8.6	8.5	8.3	0.2	0.2	0.2
				NH_4-N extracted from soil, ppm.					
1	1.2	2.1	2.3	1.2	0.2	0.1	0.1	0.1	0.1
2	3.2	3.7	1.9	0.1	0.1	0.2	0.1	0.1	0.1
3	1.4	2.2	1.4	0.1	0.4	0.8	0.1	0.1	0.1
4	0.5	1.2	2.5	0.8	0.8	0.1	0.1	0.1	0.1
Ave.	1.6	2.3	2.0	0.5	0.4	0.3	0.1	0.1	0.1
				NO_3-N extracted from soil, ppm.					
1	0.7	0.1	0.2	0.5	0.1	0.9	1.1	0.1	0.7
2	0.1	0.2	0.3	1.3	0.7	0.2	0.1	0.6	0.1
3	0.1	0.1	0.3	0.4	0.1	0.4	0.1	0.3	0.1
4	0.1	0.1	0.2	0.5	0.6	0.2	1.0	0.9	0.1
Ave.	0.3	0.2	0.3	0.7	0.4	0.4	0.6	0.3	0.3
				SO_4-S extracted from soil, ppm.					
1	0.4	0.5	0.3	0.5	0.3	0.3	0.1	0.2	0.8
2	0.8	0.4	3.6	1.7	0.4	0.4	0.2	1.1	2.2
3	1.4	0.6	0.4	1.5	0.9	1.1	0.2	1.8	0.6
4	0.8	0.9	0.4	0.8	0.1	0.4	0.1	0.2	0.2
Ave.	0.9	0.7	1.4	1.2	0.8	0.7	0.2	0.8	0.9

TABLE 7--Continued

Replicates	Zineb			Inorganic Zn, N, and S Equivalent to the Zineb			Control		
	A	B	C	A	B	C	A	B	C
	pH of soil in water after treatment								
1	5.40	5.45	5.35	4.55	4.55	4.55	5.45	5.55	5.45
2	5.45	5.40	5.45	4.55	4.50	4.55	5.45	5.45	5.35
3	5.20	5.45	5.35	4.55	4.55	4.55	5.50	5.55	5.50
4	5.55	5.35	5.45	4.55	4.55	4.60	5.45	5.50	5.55
Ave.	5.55	5.41	5.45	4.55	4.54	4.56	5.46	5.51	5.51
	pH of soil in 1 N HCl after treatment								
1	4.65	4.65	4.65	4.15	4.10	4.15	4.65	4.65	4.65
2	4.65	4.60	4.65	4.10	4.15	4.15	4.65	4.65	4.65
3	4.65	4.65	4.65	4.10	4.15	4.10	4.65	4.65	4.65
4	4.65	4.65	4.65	4.15	4.10	4.15	4.65	4.65	4.65
Ave.	4.65	4.64	4.70	4.12	4.12	4.14	4.65	4.65	4.65

APPENDIX

TABLE 8

Evolution of CO_2 where zineb, equivalent inorganic salts and sucrose, and control only were present in Pomello fine sand where A is not inoculated, B inoculated with extract from Leon fine sand and C inoculated with extract from Arredondo fine sand

Replicates	Zineb			Inorganic Zn, N, S and C Equivalent to the Zineb			Control		
	A	B	C	A	B	C	A	B	C
	CO_2-C evolved, mg.								
1	1.9	1.9	1.2	2.2	2.1	2.3	1.8	2.6	2.6
2	2.4	2.6	1.6	2.4	2.6	2.4	2.5	2.6	2.5
3	1.3	1.7	1.2	0.9	2.6	1.8	2.1	2.5	2.2
4.	1.6	2.0	1.1	1.4	1.9	2.2	2.5	2.4	2.0
Ave.	1.8	2.1	1.4	1.7	2.1	2.2	2.2	2.5	2.3

APPENDIX

TABLE 9

Results of second microbiological study on the degradation of zineb with comparisons using equivalent inorganic salts or nabam and including a Cu variable

Reps.	Treatments*									
	1	2	3	4	5	6	7	8	9	10
	Zn extracted from soil, ppm.									
1	0.2	6.6	18.0	26.7	22.8	6.9	17.0	0.2	11.3	0.1
2	0.2	8.7	22.9	26.9	21.9	7.5	28.6	0.1	15.4	0.1
3	0.2	10.2	18.8	30.0	19.4	5.7	21.0	0.1	19.0	0.2
4	0.2	4.5	12.3	18.7	23.0	7.4	27.7	0.2	9.3	0.1
Avg.	0.2	7.5	18.0	25.5	21.8	6.9	23.6	0.2	13.8	0.1
	NH_4-N extracted from soil, ppm.									
1	5.0	7.9	4.5	5.3	6.2	6.1	4.6	8.7	4.8	1.5
2	8.7	14.1	5.3	5.3	5.8	6.8	6.1	10.0	11.2	1.7
3	5.4	14.8	5.9	8.0	6.9	8.4	8.4	4.6	4.6	1.3
4	4.8	6.9	8.4	6.1	4.6	8.7	5.0	6.1	6.8	1.6
Avg	6.0	10.9	6.0	6.2	6.0	7.5	6.0	7.4	7.0	1.6
	NO_3-N extracted from soil, ppm.									
1	0.3	0.1	0.7	0.6	0.5	0.7	0.6	2.1	0.9	0.1
2	0.3	0.1	0.6	0.4	1.7	1.3	1.0	2.3	0.7	0.4
3	0.4	0.4	0.6	2.4	0.7	0.4	0.6	4.2	0.6	0.3
4	0.5	0.5	0.9	0.9	0.5	0.9	0.8	0.1	0.3	1.1
Avg	0.3	0.4	0.7	1.1	0.8	0.8	0.8	2.2	0.6	0.5
	SO_4-S extracted from soil, ppm.									
1	0.1	0.3	0.4	4.5	0.4	0.8	5.8	2.2	7.8	0.1
2	0.3	0.2	1.1	6.3	0.1	0.1	8.4	3.0	11.7	0.1
3	0.1	0.5	0.8	8.6	1.8	0.1	10.2	4.4	11.5	0.1
4	0.1	0.1	3.2	6.2	0.1	2.1	4.9	14.6	14.5	0.1
Avg	0.2	0.3	1.4	6.4	0.6	0.8	7.3	6.0	11.4	0.1

TABLE 9--Continued

Reps	Treatments[a]									
	1	2	3	4	5	6	7	8	9	10
				pH of soil in water after treatment						
1	2.90	2.70	3.30	3.00	3.55	2.85	3.60	4.55	3.60	5.20
2	2.75	2.70	3.05	3.35	3.40	2.75	3.15	4.50	4.05	4.90
3	2.70	2.75	3.00	3.35	3.40	2.90	3.40	4.15	3.80	5.20
4	2.75	2.60	3.05	3.25	3.35	3.00	3.55	2.75	3.55	5.20
Avg.	2.77	2.69	3.10	3.24	3.42	2.88	3.42	3.94	3.75	5.12
				pH of soil in 1 N KCl after treatment						
1	2.75	2.60	3.15	2.90	3.45	2.75	3.45	3.95	3.45	4.45
2	2.65	2.60	3.00	3.25	3.35	2.70	3.05	4.05	3.80	4.25
3	2.60	2.60	2.95	3.25	3.30	2.85	3.35	3.80	3.60	4.40
4	2.60	2.50	2.95	3.10	3.25	2.80	3.45	2.60	3.40	4.40
Avg.	2.65	2.58	3.01	3.12	3.34	2.78	3.32	3.60	3.56	4.38

[a]See table 2 for the treatment

APPENDIX

TABLE 10

CO_2 evolution from Pomello fine sand at different intervals in the second microbiological study on the degradation of zineb with companions using equivalent inorganic salts, or nabam, and including a Cu variable

Treatment[a]	Days Elapsed from Initiation of Experiment							
	4	10	17	24	31	34	38	45
1	9.1	13.2	42.8	30.9	43.3	19.9	35.8	31.5
	10.1	14.0	43.7	40.1	42.9	18.8	43.0	45.8
	10.0	13.6	41.4	37.2	43.8	10.3	43.2	45.1
	9.1	12.8	44.6	39.2	42.5	20.2	37.8	43.8
Avg.	9.6	13.4	43.1	36.8	43.1	19.6	40.0	41.6
2	7.1	10.3	37.7	35.8	26.3	25.8	41.3	41.4
	2.8	10.5	32.2	21.6	17.4	16.9	19.0	22.8
	3.1	17.3	25.8	14.8	7.9	20.1	29.2	9.7
	4.1	11.0	31.2	26.0	30.4	9.2	38.5	37.0
Avg.	4.3	12.3	31.8	24.6	20.5	18.0	32.0	27.7
3	30.8	22.2	23.5	25.2	25.0	26.7	20.5	24.2
	33.7	21.3	26.3	25.7	30.7	16.8	24.3	25.9
	32.3	22.8	23.7	20.8	30.4	31.3	27.1	29.2
	32.0	22.7	30.4	25.3	27.1	25.6	25.6	25.4
Avg.	32.2	22.2	26.0	23.0	28.3	25.1	24.4	26.2
4	8.9	31.1	30.1	27.6	25.9	12.7	23.3	28.3
	7.1	31.0	18.2	19.0	24.0	43.4	26.0	26.8
	7.7	32.6	26.6	19.5	26.5	7.7	28.1	26.8
	4.6	31.1	27.5	20.8	27.7	9.4	17.8	33.1
Avg.	7.2	31.4	28.1	21.7	26.0	18.3	23.8	28.2
5	30.5	20.6	21.7	14.6	18.5	17.9	14.6	12.1
	29.9	20.4	20.8	11.8	20.7	37.0	18.3	19.7
	29.9	23.2	21.3	15.0	35.8	14.8	25.2	18.2
	29.7	20.7	21.7	13.4	19.9	13.3	25.7	21.4
Avg.	30.0	21.2	21.4	13.7	23.7	20.8	20.4	17.8
6	5.5	6.8	16.8	17.3	27.0	33.8	29.4	31.0
	4.5	6.5	13.2	15.7	20.1	20.0	25.8	25.2
	4.7	6.7	14.2	17.3	26.4	37.0	24.0	31.0
	4.9	5.8	8.7	5.0	10.1	12.8	14.1	12.8
Avg.	4.8	6.4	13.2	13.8	20.9	25.9	23.3	25.0
7	23.1	20.8	24.0	19.0	27.2	27.7	26.3	27.6
	24.0	28.8	24.8	18.5	30.2	27.5	18.7	35.1
	18.0	29.3	24.8	14.8	25.1	9.9	16.4	17.5
	17.9	30.8	21.8	16.2	22.2	11.7	22.3	23.1
Avg.	20.8	27.4	23.8	17.1	26.2	19.2	20.9	25.8

TABLE 10--Continued

Treatment[a]	Days Elapsed from Initiation of Experiment							
	4	10	17	24	31	34	38	45
8	0.3	0.5	9.7	12.0	13.3	13.6	13.5	14.4
	0.1	0.1	8.1	10.3	10.3	22.5	10.3	10.9
	0.8	3.6	11.0	10.4	12.2	25.7	14.8	11.4
	1.3	7.4	10.7	13.0	21.3	39.3	23.9	26.4
Avg.	0.6	2.8	9.9	11.4	14.2	25.3	15.6	15.8
9	0.2	10.2	13.0	21.2	22.7	23.8	21.6	24.0
	0.2	0.1	1.2	8.2	16.7	27.0	21.4	21.6
	1.0	15.6	29.0	19.8	20.5	13.5	22.5	16.9
	0.7	7.7	28.3	24.4	25.8	29.8	26.7	26.4
Avg.	0.5	8.4	17.8	18.4	21.4	24.0	23.0	22.2
10	1.4	1.8	1.7	1.2	1.3	2.4	5.9	5.5
	0.7	1.5	1.7	2.8	1.2	0.1	0.9	1.4
	1.5	2.8	2.3	1.2	1.7	1.9	3.2	1.8
	1.3	1.5	6.2	1.8	1.7	2.2	2.1	1.5
Avg.	1.2	1.9	3.0	1.8	1.5	1.6	3.0	2.6

[a]See table 2 in text for treatments.

APPENDIX

TABLE 11

Effects of Mg addition on the growth and chemical composition of sour orange seedlings grown with and without zineb in unlimed Arredondo fine sand

Replicates	Mg Addition			
	None		65 lb./A.	
	Zineb Addition			
	None	32 lb./A.	None	32 lb./A.
Green weight of new foliage, g.				
1	53.5	65.4	65.9	56.3
2	60.8	62.4	56.3	69.8
3	64.5	61.3	61.0	71.4
4	56.0	56.3	60.7	60.6
Avg.	58.7	61.8	61.0	64.0
Green weight of leaves, g.				
1	37.2	41.5	40.1	32.8
2	33.6	39.8	36.2	43.3
3	41.5	39.8	39.2	44.9
4	31.6	36.2	37.3	38.3
Avg.	36.0	39.3	37.7	39.8
Dry weight of leaves, g.				
1	15.3	16.7	16.3	15.5
2	13.2	15.4	15.4	18.5
3	15.2	17.2	17.1	17.8
4	12.7	14.2	16.1	16.8
Avg.	16.1	15.8	16.2	16.6
Dry weight of roots, g.				
1	15.4	15.9	15.8	15.3
2	14.6	14.4	14.4	16.8
3	13.5	13.0	14.8	16.7
4	11.8	10.5	16.5	10.1
Avg.	15.3	15.3	33 15.8	16.1

TABLE 11--Continued

Replicates	Mg Addition: None		Mg Addition: 65 lb./A.	
	Zineb Addition: None	Zineb Addition: 32 lb./A.	Zineb Addition: None	Zineb Addition: 32 lb./A.
Ca content of leaves, %				
1 1	1.89	1.91	2.07	2.49
2	2.30	2.19	2.61	1.96
3	2.21	2.03	1.95	2.16
4	2.04	2.09	2.22	1.67
Avg.	2.11	2.08	2.21	2.07
P content of leaves, %				
1	0.17	0.15	0.18	0.19
2	0.16	0.17	0.17	0.16
3	0.19	0.17	0.15	0.17
4	0.17	0.19	0.18	0.17
Avg.	0.17	0.17	0.17	0.17
Mg content of leaves, %				
1	0.26	0.30	0.28	0.24
2	0.26	0.30	0.26	0.23
3	0.24	0.21	0.26	0.23
4	0.23	0.24	0.26	0.22
Avg.	0.25	0.26	0.26	0.23
K content of leaves, %				
1	1.10	0.96	1.09	1.09
2	1.02	1.04	0.63	0.63
3	1.08	1.08	1.04	1.04
4	0.92	1.09	0.96	0.96
Avg.	1.03	1.04	0.93	0.93

TABLE 11--Continued

Replicates	Mg Addition			
	None		65 lb./A	
	Zineb Addition			
	None	32 lb./A	None	32 lb./A
Ca content of roots, %				
1	0.45	0.66	1.14	0.51
2	0.81	0.60	0.45	0.87
3	0.78	0.45	0.45	0.48
4	1.17	0.87	0.39	1.32
Avg.	0.80	0.65	0.61	0.80
P content of roots, %				
1	0.17	0.18	0.16	0.17
2	0.22	0.18	0.17	0.15
3	0.22	0.17	0.15	0.18
4	0.15	0.17	0.19	0.18
Avg.	0.19	0.18	0.18	0.16
Mg content of roots, %				
1	0.27	0.27	0.27	0.27
2	0.30	0.39	0.24	0.27
3	0.36	0.30	0.33	0.24
4	0.33	0.30	0.30	0.33
Avg.	0.38	0.30	0.30	0.27
K content of roots, %				
1	1.41	1.20	1.44	1.74
2	1.50	1.65	2.28	1.47
3	1.77	1.38	2.28	1.29
4	1.26	1.59	1.50	2.13
Avg.	1.50	1.44	1.86	1.65

TABLE 11--Continued

Replicates	Mg Addition			
	None		65 lb./A	
	Zineb Addition			
	None	32 lb./A	None	32 lb./A
	Zn content of leaves, ppm			
1	8.5	7.9	8.8	10.2
2	8.8	10.3	8.5	9.0
3	13.3	9.3	11.8	9.1
4	6.8	8.4	8.1	14.6
Avg.	9.2	9.0	9.3	10.7
	Cu content of leaves, ppm			
1	3.7	3.4	2.7	3.5
2	4.5	2.7	3.4	3.0
3	3.3	3.0	3.7	7.6
4	2.0	3.0	2.9	3.0
Avg.	3.4	3.0	3.2	3.0
	Mn content of leaves, ppm			
1	20.3	26.3	16.0	25.2
2	18.2	28.6	24.4	16.8
3	24.7	20.9	15.2	20.8
4	18.9	22.5	21.1	22.0
Avg.	20.5	24.6	19.2	21.2
	Fe content of leaves, ppm.			
1	74	39	40	50
2	54	71	34	30
3	57	50	32	50
4	49	72	55	53
Avg.	58	58	40	46

TABLE 11--Continued

Replicates	Mn Addition			
	None		65 lb./A	
	Zineb Addition			
	None	32 lb./A.	None	32 lb./A
Cu content of roots, ppm				
1	7.2	7.2	5.8	7.3
2	7.5	6.5	7.2	5.9
3	7.7	6.2	6.4	5.4
4	5.8	7.2	6.7	8.0
Avg.	7.0	6.8	6.5	6.6
Mn content of roots, ppm.				
1	33	33	28	31
2	33	32	40	31
3	31	34	34	27
4	33	34	35	39
Avg	32	33	34	32
Fe content of roots, ppm.				
1	1,524	1,720	1,336	1,651
2	1,888	1,642	1,574	1,292
3	1,795	1,574	1,790	1,177
4	1,081	1,096	1,601	1,458
Avg.	1,572	1,508	1,575	1,395

APPENDIX

TABLE 12

Effect of Zn from two sources and Mg addition to two soils on the growth, leaf composition and root composition of sour orange seedlings

	Soil Type							
	Arredondo fine sand				Leon fine sand			
	Zinc Source at 5 Pounds per Acre							
Reps.	Zineb		$ZnSO_4$		Zineb		$ZnSO_4$	
	Magnesium Source, Pounds per Acre							
	0	65	0	65	0	65	0	65
	Green weight of new foliage, g.							
1	44	42	57	48	42	37	40	44
2	47	55	46	44	33	39	39	43
3	43	43	44	39	29	37	37	38
Avg.	44	46	49	43	35	37	39	42
	Number of new leaves							
1	33	43	53	55	39	32	46	50
2	37	39	35	47	30	32	34	47
3	42	52	32	53	32	35	38	29
Avg.	37	44	40	52	34	33	38	42
	Dry weight of new leaves, g.							
1	12.5	12.7	15.1	13.8	13.0	11.2	9.8	12.7
2	14.3	15.4	13.2	13.5	10.2	11.4	11.1	10.7
3	14.1	13.0	12.5	13.7	8.9	10.4	11.1	11.3
Avg.	13.6	13.7	13.6	13.8	10.7	11.0	10.7	11.6
	Ca content of leaves, %							
1	3.98	3.23	3.41	3.00	2.22	2.22	1.92	1.98
2	3.15	3.82	3.34	2.93	2.20	2.73	2.27	2.39
3	3.24	3.17	3.14	3.25	2.47	2.46	1.98	2.12
Avg.	3.42	3.40	3.30	3.06	2.50	2.47	2.08	2.16

TABLE 12--Continued

	Soil Type							
	Arredondo fine sand				Leon fine sand			
	Zinc Source at 3 Pounds per Acre							
Reps.	Zineb		$ZnSO_4$		Zineb		$ZnSO_4$	
	Magnesium Source, Pounds per Acre							
	0	65	0	65	0	65	0	65
	P content of leaves, %							
1	0.20	0.18	0.18	0.19	0.18	0.18	0.17	0.18
2	.14	.17	.13	.19	.18	.17	.18	.20
3	.21	.18	.18	.15	.20	.20	.17	.20
Avg.	0.19	0.18	0.18	0.18	0.18	0.19	0.17	0.19
	Mg content of leaves, %							
1	0.44	0.34	0.40	0.43	0.42	0.42	0.44	0.40
2	.38	.35	.35	.30	.50	.49	.43	.52
3	.38	.35	.35	.30	.49	.36	.44	.52
Avg.	0.60	0.35	0.36	0.34	0.47	0.42	0.44	.48
	K content of leaves, %							
1	1.07	0.90	1.27	0.98	0.97	1.03	1.27	0.94
2	.94	.94	.81	1.00	1.13	1.01	1.14	1.26
3	.96	.98	1.09	.89	1.12	1.08	1.23	1.15
Avg.	0.99	0.91	1.06	0.96	1.07	1.04	1.21	1.15
	Zn content of leaves, ppm.							
1	15.6	13.9	26.0	12.2	18.1	21.0	15.8	14.4
2	11.2	9.9	15.5	18.0	13.4	12.4	14.8	18.4
3	15.2	11.6	18.4	10.3	13.5	40.9	12.7	15.6
Avg.	14.0	11.7	20.0	13.5	15.0	24.8	14.4	16.8

TABLE 12--Continued

	Soil Type							
	Arredondo fine sand				Leon fine sand			
	Zinc Source at 5 Pounds per Acre							
Reps.	Zineb		$ZnSO_4$		Zineb		$ZnSO_4$	
	Magnesium Source, Pounds per Acre							
	0	65	0	65	0	65	0	65
	Cu content of roots, ppm.							
1	8.7	18.2	8.1	14.7	18.1	17.7	18.3	14.7
2	7.3	7.1	6.2	7.2	17.5	16.6	15.1	14.1
3	6.7	8.7	8.2	7.9	16.3	14.0	17.1	18.6
Avg.	7.6	11.3	7.5	9.9	17.3	16.1	16.8	15.8
	Mn content of roots, ppm.							
1	36	76	38	39	25	23	25	24
2	35	41	26	33	28	21	15	19
3	40	38	38	41	32	16	18	35
Avg.	37	52	34	38	28	20	19	26
	Fe content of roots, ppm.							
1	1,810	3,210	2,020	2,190	175	169	293	242
2	1,820	2,340	1,420	1,990	448	219	147	182
3	2,140	2,020	1,860	1,810	161	164	120	244
Avg.	1,923	2,523	1,766	1,996	261	184	187	223
	Dry weight of roots, g.							
1	8.9	7.8	10.6	10.0	10.1	8.9	9.0	10.1
2	9.0	11.4	10.4	12.4	12.9	9.6	12.2	9.5
3	10.2	13.7	9.4	13.2	9.6	9.2	8.9	10.4
Avg.	9.4	11.0	10.1	11.9	10.9	9.1	10.0	10.0

TABLE 12.--Continued

	Soil Type							
	Arredondo fine sand				Leon fine sand			
	Zinc Source at 5 Pounds per Acre							
Reps.	Zineb		$ZnSO_4$		Zineb		$ZnSO_4$	
	Magnesium Source, Pounds per Acre							
	0	65	0	65	0	65	0	65
	Cu content of leaves, ppm.							
1	4.6	3.5	5.1	5.4	6.9	7.2	7.4	7.7
2	4.0	4.1	4.4	4.3	6.0	7.0	7.0	9.4
3	4.5	3.7	4.6	4.5	6.5	7.4	7.0	6.9
Avg.	4.4	5.8	4.7	4.7	6.5	7.2	7.1	8.0
	Mn content of leaves, ppm.							
1	9.5	10.0	10.1	9.5	6.4	6.0	7.4	6.5
2	8.4	9.0	8.2	8.2	5.5	7.3	5.9	4.4
3	7.2	10.1	9.6	8.8	8.2	7.0	8.1	7.3
Avg.	8.4	9.7	9.3	8.8	6.7	6.8	7.1	6.1
	Fe content of leaves, ppm.							
1	41	41	25	45	35	37	43	27
2	12	50	16	49	39	41	44	43
3	46	46	21	44	45	33	34	42
Avg.	33	46	21	46	40	37	40	37
	Ca content of roots, %							
1	1.00	0.72	0.49	0.90	1.30	1.25	1.15	1.35
2	0.75	0.96	0.63	0.70	1.15	1.50	1.00	1.55
3	0.50	0.36	0.59	0.69	1.12	1.30	1.21	1.40
Avg.	0.75	0.68	0.57	0.76	1.19	1.35	1.12	1.43

TABLE 12--*Continued*

	Soil Type							
	Arredondo fine sand				Leon fine sand			
	Zinc Source at 5 Pounds per Acre							
Reps.	Zineb		$ZnSO_4$		Zineb		$ZnSO_4$	
	Magnesium Source, Pounds per Acre							
	0	65	0	65	0	65	0	65
	P content of roots, etc.							
1	.18	.27	.16	.17	.13	.12	.14	.13
2	.17	.20	.12	.18	.10	.12	.10	.09
3	.16	.17	.16	.15	.10	.11	.12	.10
Avg	0.17	0.21	0.15	0.17	0.11	0.12	0.12	0.16
	Mg content of roots, %							
1	0.72	1.88	0.89	1.00	1.21	1.06	1.13	1.20
2	0.78	0.95	0.67	0.96	0.68	1.10	1.12	1.06
3	0.84	0.94	0.96	0.82	1.09	1.06	1.22	1.37
Avg.	0.78	1.26	0.84	0.93	0.99	1.07	1.16	1.21
	K content of roots, %							
1	1.15	1.40	0.88	1.05	1.19	0.59	1.25	1.06
2	1.06	0.95	0.90	0.95	1.04	1.12	1.17	0.84
3	1.10	1.07	1.06	1.06	1.17	1.08	1.38	1.18
Avg.	1.11	1.14	0.95	1.01	1.13	0.93	1.27	1.03
	Zn content of roots, ppm.							
1	38	97	27	52	196	88	113	55
2	55	33	21	26	69	69	65	59
3	73	31	34	25	72	70	67	70
Avg.	55	54	27	34	112	76	81	61

APPENDIX

TABLE 13

Effect of Cu addition on the growth and chemical composition of sour orange seedlings grown on limed Arredondo fine sand with or without zineb at the rate of 32 pounds per acre or the equivalent nabam

Replicates	Cu Addition			
		200 lb./A.		
		Dithiocarbamate Addition		
	None	None	Zineb	Nabam
	Green weight of foliage, g.			
1	70.4	83.0	67.4	76.5
2	83.7	92.5	77.8	84.7
3	84.3	87.6	92.8	105.2
4	83.9	97.4	71.3	92.5
Avg.	80.6	90.1	77.6	89.6
	Green weight of leaves, g.			
1	43.7	50.8	44.2	47.8
2	51.3	56.2	55.2	53.0
3	51.4	54.6	57.8	65.6
4	50.9	60.1	48.2	58.7
Avg.	49.1	55.3	51.4	56.3
	Dry weight of leaves, g			
1	14.7	17.9	15.3	16.5
2	18.8	20.9	16.0	19.2
3	17.2	17.4	18.3	25.4
4	16.2	21.5	18.3	23.5
Avg.	16.7	19.4	17.0	21.1
	Dry weight of roots, g.			
1	15.0	20.1	15.6	18.8
2	21.5	22.8	16.5	17.8
3	20.8	13.7	16.8	25.3
4	14.6	21.6	22.5	23.2
Avg.	18.3	19.9	18.2	21.6

TABLE 13--Continued

Replicates	Cu Addition			
		200 lb./A		
		Dithiocarbamate Addition		
	None	None	Zineb	Nabam
	Ca content of leaves, %			
1	2.38	2.60	2.43	2.54
2	2.45	2.23	2.47	2.45
3	2.43	2.51	2.44	2.55
4	2.30	2.51	2.44	2.44
Avg.	2.39	2.46	2.44	2.50
	P content of leaves, %			
1	0.21	0.18	0.18	0.20
2	0.17	0.19	0.18	0.20
3	0.20	0.20	0.20	0.16
4	0.22	0.17	0.18	0.16
Avg.	0.20	0.18	0.18	0.18
	Mg content of leaves, %			
1	0.28	0.29	0.27	0.28
2	0.25	0.24	0.29	0.26
3	0.27	0.28	0.28	0.24
4	0.27	0.29	0.28	0.25
Avg.	0.27	0.28	0.28	0.26
	K content of leaves, %			
1	0.96	0.83	0.85	0.66
2	0.74	0.77	0.77	0.72
3	0.93	0.92	0.87	0.67
4	0.93	0.83	0.74	0.79
Avg	0.88	0.83	0.81	0.71

TABLE 13--Continued

Replicates	Cu Addition			
		200 lb./A.		
		Dithiocarbamate Addition		
	None	None	Zineb	Nabam
Zn content of leaves, ppm.				
1	16.5	14.2	14.6	13.4
2	14.0	15.7	14.2	15.3
3	15.5	20.3	19.7	15.6
4	19.2	13.0	13.2	13.7
Avg.	16.3	15.8	15.3	14.5
Cu content of leaves, ppm				
1	12.3	12.3	11.2	16.5
2	11.8	12.5	11.6	13.2
3	14.3	12.8	13.4	13.9
4	11.2	12.4	15.7	14.3
Avg.	12.4	12.5	13.0	14.5
Mn content of leaves, ppm.				
1	21.1	24.6	21.5	22.5
2	25.0	17.3	23.1	22.4
3	20.9	17.0	29.5	26.8
4	17.9	20.5	25.7	24.7
Avg.	21.2	19.9	25.0	24.1
Fe content of leaves, ppm.				
1	27.6	38.9	43.9	51.1
2	52.5	47.3	37.0	47.5
3	24.4	47.9	54.8	37.3
4	39.9	51.2	45.7	25.3
Avg.	36.1	46.3	45.3	40.3

TABLE 13--Continued

Replicates	Cu Addition			
		200 lb./A		
		Dithiocarbamate Addition		
	None	None	Zineb	Nabam
	Ca content of roots, %			
1	0.81	0.69	0.73	0.90
2	0.99	1.11	0.73	0.99
3	0.84	0.99	1.05	0.87
4	1.02	0.81	0.75	0.73
Avg.	0.92	0.90	0.91	0.87
	P content of roots, %			
1	0.14	0.18	0.16	0.16
2	0.15	0.11	0.17	0.19
3	0.19	0.16	0.18	0.15
4	0.18	0.16	0.15	0.17
Avg.	0.16	0.13	0.16	0.17
	Mg content of roots, %			
1	0.42	0.33	0.24	0.27
2	0.30	0.30	0.45	0.42
3	0.40	0.66	0.27	0.36
4	0.32	0.27	0.52	0.39
Avg.	0.36	0.40	0.36	0.36
	K content of roots, %			
1	0.45	0.75	0.84	0.69
2	0.69	0.66	0.90	0.84
3	0.72	0.74	0.96	0.57
4	0.81	0.72	0.66	0.63
Avg.	0.66	0.72	0.84	0.69

TABLE 13--Continued

Replicates	Cu Addition			
		200 lb./A		
		Dithiocarbamate Addition		
	None	None	Zineb	Nabam
	Zn content of roots, ppm			
1	93	67	71	44
2	59	70	45	44
3	45	36	58	46
4	35	64	61	66
Avg.	57	59	59	50
	Cu content of roots, ppm			
1	43	46	43	45
2	38	52	47	53
3	47	53	89	47
4	38	68	48	72
Avg	41	54	55	55
	Mn content of roots, ppm.			
1	49	68	61	39
2	59	56	57	59
3	61	61	60	51
4	51	63	56	62
Avg.	54	61	58	52
	Fe content of roots, ppm			
1	526	529	407	443
2	297	611	484	555
3	628	463	827	578
4	497	627	552	692
Avg.	480	548	551	558

APPENDIX

TABLE 14

Analysis of Zn and Cu in leachates from limed Arredondo fine sand with and without copper alone or with zineb or nabam treatments

Treatment	Replicates	Leachates 1	2	3	4	5	Total
		Zn					
		mg. x 10^{-3}					mg.
Check	1	2.8	1.2	3.8	2.4	1.3	0.338
	2	2.0	2.8	4.6	5.4	3.1	0.518
	3	1.8	1.5	3.7	3.3	1.3	0.314
	4	8.6	0.8	2.0	1.8	0.2	0.424
	Ave.	3.8	1.4	3.5	3.2	1.5	0.398
Cu	1	5.5	7.5	4.2	3.2	1.2	0.549
	2	2.9	0.6	1.4	2.0	1.0	0.205
	3	2.5	0.7	3.5	2.3	1.3	0.193
	4	0.1	0.6	3.0	2.1	1.5	0.190
	Ave.	2.8	2.2	3.0	2.4	1.3	0.284
Cu plus zineb	1	2.9	1.7	3.6	2.9	1.7	0.423
	2	1.1	0.4	2.0	2.4	2.0	0.217
	3	2.2	0.3	2.2	1.8	0.8	0.213
	4	2.8	2.1	4.2	2.7	1.0	0.359
	Ave.	2.3	1.1	3.0	2.4	1.4	0.303
Cu plus nabam	1	2.4	0.6	1.8	2.3	4.8	0.392
	2	3.8	1.7	3.4	3.0	4.2	0.468
	3	1.0	1.2	2.7	2.5	1.3	0.260
	4	1.8	1.2	3.7	2.6	1.4	0.260
	Ave.	2.2	1.2	2.9	2.6	2.9	0.330

TABLE 14--Continued

Treatment	Replicates	Leachates					
		1	2	3	4	5	Total
		Cu					
		mg. x 10^{-3}					
Check	1	12.5	14.2	12.5	10.8	6.2	1.575
	2	11.2	14.2	12.7	12.5	8.0	1.802
	3	10.8	14.4	11.5	12.3	7.5	1.473
	4	11.5	12.5	10.6	10.6	6.9	1.543
	Ave.	11.5	12.8	11.7	11.8	7.2	1.598
Cu	1	10.0	12.5	16.1	11.5	6.2	1.517
	2	9.5	12.0	12.5	12.0	6.2	1.439
	3	11.7	12.5	12.6	12.9	7.5	1.439
	4	9.2	13.8	12.1	12.0	7.5	1.460
	Ave.	10.1	13.1	13.8	12.1	6.8	1.464
Cu plus zineb	1	10.0	12.5	11.8	11.5	6.9	1.732
	2	11.5	12.0	9.8	12.0	8.6	1.508
	3	8.5	12.0	11.3	11.7	7.5	1.465
	4	8.6	10.6	12.7	11.2	8.0	1.405
	Ave.	9.7	11.8	11.4	11.6	7.8	1.527
Cu plus Nabam	1	11.5	11.2	12.5	12.3	7.3	1.725
	2	14.2	12.5	12.7	13.5	6.7	1.806
	3	12.5	11.7	12.1	10.8	6.9	1.596
	4	11.5	12.5	12.6	11.5	6.9	1.437
	Ave.	12.4	12.7	12.9	12.5	7.0	1.641

TABLE 14--Continued

Treatment	Replicates	Leachates 1	2	3	4	5
		pH				
Check	1	7.50	8.55	8.15	8.35	8.25
	2	7.70	8.45	8.05	8.15	8.25
	3	8.80	8.55	8.20	8.25	8.35
	4	7.35	8.50	8.25	8.80	8.40
	Ave.	7.34	8.51	8.16	8.26	8.31
Cu	1	7.40	8.40	8.00	8.30	8.30
	2	7.40	8.55	8.15	8.25	8.35
	3	7.45	8.50	8.10	8.35	8.30
	4	7.25	8.50	8.15	8.25	8.30
	Ave.	7.38	8.49	8.10	8.29	8.31
Cu plus zineb	1	7.40	8.45	8.00	8.15	8.25
	2	7.55	8.55	8.35	8.25	8.30
	3	7.85	8.45	8.20	8.30	8.36
	4	7.85	8.50	8.20	8.25	8.35
	Ave.	7.66	8.49	8.19	8.24	8.32
Cu plus nabam	1	7.70	8.55	8.15	8.10	8.20
	2	7.50	8.40	8.05	8.05	8.30
	3	7.85	8.55	8.15	8.25	8.35
	4	8.00	8.65	8.30	8.25	8.35
	Ave.	7.76	8.54	8.16	8.16	8.30

APPENDIX

TABLE 15

The vertical distribution of Zn after completion of the second Mg experiment

Soil Type	Replicates	Zineb				$ZnSO_4$			
		Increment of Soil Sample, in.							
		1	2	3	4	1	2	3	4
Arredondo	1	12.6	3.2	2.3	2.9	13.0	2.5	5.6	3.1
	2	21.0	5.2	4.0	2.9	15.6	6.2	2.3	2.0
	3	15.0	6.5	2.7	3.2	10.0	2.0	1.8	2.0
	Ave.	16.2	5.6	3.0	3.0	12.9	3.6	3.2	2.4
Leon	1	15.6	2.9	3.6	3.1	15.0	2.5	5.6	3.1
	2	16.8	4.4	2.3	3.3	15.6	6.2	2.3	2.0
	3	14.6	2.5	2.4	2.5	10.0	2.0	1.6	2.0
	Ave.	15.7	3.3	2.7	3.0	12.9	3.6	3.2	2.7

BIOGRAPHICAL SKETCH

James Richard Iley was born April 26, 1930, in Plant City, Florida, the son of William David Iley and Frances Wren Lemoreaux Iley. He attended Plant City High School in Plant City, Florida, graduating in June, 1948. After graduating from high school he was engaged in self-employed agricultural activities.

In September, 1950, he entered Florida Southern College, Lakeland, Florida, where he received the degree of Bachelor of Science in Chemistry in June, 1954. He was employed by the Department of Agronomy, Florida Agricultural Experiment Station, University of Florida, Gainesville, Florida, from July, 1954, until October, 1955. From October, 1955, to September, 1959, he was employed as a chemist for the American Agricultural Chemical Company at Pierce, Florida. He attended the Artillery and Missile School at Ft. Sill, Oklahoma, from September, 1959, until February, 1960. In February, 1960, he entered the University of Florida, Gainesville, Florida, and is now a candidate for the degree of Doctor of Philosophy.

He is a member of the agricultural honorary fraternity Gamma Sigma Delta. He is a member of the American Chemical Society, the American Society of Agronomy, and the American Society of Plant Physiologists.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

April 20, 1963

S. P. Thornton
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